REPLAGAL™

(agalsidase alfa)

BRIEFING DOCUMENT

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Prepared by TKT, Inc for the

Endocrinologic and Metabolic Drugs

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1. EXECUTIVE SUMMARY

- Replagal is safe and effective for the treatment of patients with Fabry Disease.

- Fabry Disease is a rare X-linked inherited metabolic disorder caused by deficient activity of the lysosomal enzyme α-galactosidase A. Fabry Disease is a complex clinical syndrome that includes severe renal, cardiac, and neurological manifestations. The pathophysiology of Fabry Disease is well established: due to a lack of the lysosomal enzyme α-galactosidase A, there is accumulation of globotriaosylceramide (Gb_3) throughout the body. Throughout the course of the disease, there is progressive damage to the following cell types which leads to the principal clinical manifestations of Fabry Disease:

  - In the kidney, the entire glomerulus becomes extensively damaged. Specifically, damage to the renal glomerular epithelial cells (podocytes) and glomerular mesangial cells leads to expansion of the mesangial matrix (mesangial widening), focal and segmental glomerular sclerosis, and ultimately glomerular obsolescence. Glomerular damage leads to chronic renal insufficiency and End Stage Renal Disease (ESRD). Renal failure is the leading cause of death in Fabry Disease.

  - In the heart, cardiac myocytes are damaged which leads to hypertrophic cardiomyopathy and ultimately myocardial infarction, arrythmias, and valvular dysfunction.

  - The mean age at which patients with Fabry Disease progress to ESRD is approximately age 38. Death typically occurs in the fourth or fifth decade of life.

  - There is no approved therapy for Fabry Disease in the United States. Currently therapy is limited to palliation of end-organ damage with non-specific modalities (eg, dialysis and pain medication).

- Replagal (agalsidase alfa) is a human α-galactosidase A produced by genetic engineering technology in a human cell line. As a result, Replagal has a fully human glycosylation profile that ensures appropriate biodistribution and uptake by clinically relevant target cells. In addition, the absence of non-human glycosylation minimizes immunogenicity.

- Replagal is administered at a dose of 0.2 mg/kg every other week by intravenous infusion over 40 minutes.

- Replagal improves the kidney pathology of Fabry Disease.

  - In standard glomerular histopathology measurements Replagal increases the fraction of glomeruli that are normal and decreases the fraction of glomeruli that have mesangial widening.

  - These measurements of standard renal histopathology correlate with renal function as measured by both GFR and creatinine clearance. Therefore these measurements are a valid surrogate marker of disease because they are reasonably likely to predict clinical benefit.

  - Measurements of Gb_3 storage (plasma levels, urine sediment levels, or deposition in the kidney) do not correlate with any measure of renal function.

- Treatment with Replagal for up to 2.5 years has demonstrated the following:

  - Initial stabilization and subsequent improvement of kidney function

  - Improvement of cardiac structure and function

  - Improvement of metabolic function

- The total number of patients who have received Replagal is in excess of 300 patients worldwide – including patients who have received commercial drug since its approval in Europe in August 2001.

- Replagal has an excellent safety profile. The incidence of infusion reactions to Replagal is less than 10%. Approximately 50% of patients who received Replagal in multidose studies developed low titer IgG antibodies. Among 40 male patients followed for up to 2.5 years, persistent IgG antibodies were observed in 11 patients (28%). Importantly, no patients treated with Replagal have developed an IgE antibody response, and there have been no adverse events that would suggest an IgE-mediated syndrome. None of the fifteen female patients treated with Replagal for 3 to 12 months developed antibodies.

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2. OVERVIEW OF REPLAGAL

Replagal (agalsidase alfa) is a human α-galactosidase A produced by genetic engineering technology in a human cell line. Agalsidase alfa is a homodimer comprised of two approximately 50 kDa subunits, with each subunit containing 398 amino acid residues. Since agalsidase alfa is synthesized by a human cell line, it not only has the identical amino acid sequence as that of α-galactosidase A produced in human tissues, but also has a fully human glycosylation profile.

While the enzymatic activity of Replagal resides in the protein portion of the molecule, glycosylation plays an essential role in its therapeutic activity. The administered enzyme must be internalized into cells and be transported to lysosomes where the toxic substrate accumulates in Fabry Disease. Furthermore, to effectively treat Fabry Disease the enzyme must be internalized into clinically relevant target cells, which include renal glomerular epithelial and mesangial cells, renal tubular epithelial cells, and cardiac myocytes. These properties (internalization and appropriate biodistribution) are controlled by the glycosylation profile of the molecule.

Replagal is targeted to its lysosomal site of action by mannose-6-phosphate (M6P) residues of the glycosylated protein. The M6P moiety binds to a specific receptor (the M6P receptor) on cell surfaces, leading to internalization and targeting to intracellular lysosomes.

Replagal is a highly purified preparation. Biological activity of Replagal is measured using the water-soluble substrate 4–methylumbelliferyl–α-D-galactopyranoside (4-MUF–gal), and biological potency is measured based on its ability to be taken up by normal human cells.

Replagal is provided as a sterile, clear and colorless solution intended for intravenous administration. The Drug Product is supplied in a single use vial.

Transkaryotic Therapies Inc. (TKT) is the sponsor of Replagal. The company was founded in 1988 and is headquartered in Cambridge, Massachusetts. TKT has over 450 employees located at its facilities in the US and Europe. TKT has a major focus in the development of biopharmaceuticals for the treatment of rare inherited diseases. In addition to Fabry Disease, ongoing development programs include therapies for Hunter syndrome and Morquio A syndrome. If approved, Replagal will be TKT's first commercial product in the United States.

3. Clinicopathological Overview of Fabry Disease

3.1. Introduction

Fabry Disease is an extremely rare X-linked inherited metabolic disorder caused by deficient activity of the lysosomal enzyme α-galactosidase A **[1,2]**. The incidence of Fabry Disease has been estimated to be approximately 1 per 117,000 births; the US population is estimated to be 1500 – 2000 patients **[6]**. Gb_3 (also known as ceramide trihexoside, CTH), the glycosphingolipid substrate for α-galactosidase A, progressively accumulates within vulnerable cells and tissues of affected patients. Renal glomerular epithelial cells (podocytes), renal glomerular mesangial cells, renal tubular epithelial cells, myocardial cells, dorsal root ganglion cells, cells of the vascular system, and cells of the autonomic nervous system are damaged by Gb_3 **[1,2,3,4,5]**.

Clinical onset of the disease typically occurs during childhood or adolescence with recurrent episodes of severe pain in the extremities, characteristic cutaneous lesions known as angiokeratomas, and a distinctive but asymptomatic corneal dystrophy. The mean age at diagnosis for males with Fabry Disease is in the range of 21.9 to 28.6 years **[6**,**20]**. Death usually occurs during the fourth or fifth decade of life from renal, cardiac, or cerebrovascular complications **[2,18].**

Fabry Disease exhibits X-linked inheritance; however, most female heterozygotes are affected clinically, indeed female heterozygotes can be as severely affected as male hemizygotes **[7,8,9]**.

There is no definitive treatment for these patients. Medical management of the renal, cardiac, and central nervous system manifestations of Fabry Disease is nonspecific and palliative. All care given to these patients is supportive in nature. The mean age at which patients with Fabry Disease progress to ESRD is approximately age 38. Dialysis and renal transplantation are utilized in patients with chronic renal failure.

The following discussion summarizes the literature in Fabry Disease with an emphasis on the correlation of pathologic findings with clinical syndromes and outcomes in Fabry Disease. In the kidney and heart there is a broad spectrum of pathology and clinical syndromes, and a continuum of pathologic changes and corresponding clinical sequelae emerges. It is reasonable to expect, based on the pathophysiology of Fabry Disease, that reversal of the pathological changes in the kidney (not simply Gb_3 removal) will lead to clinical improvement.

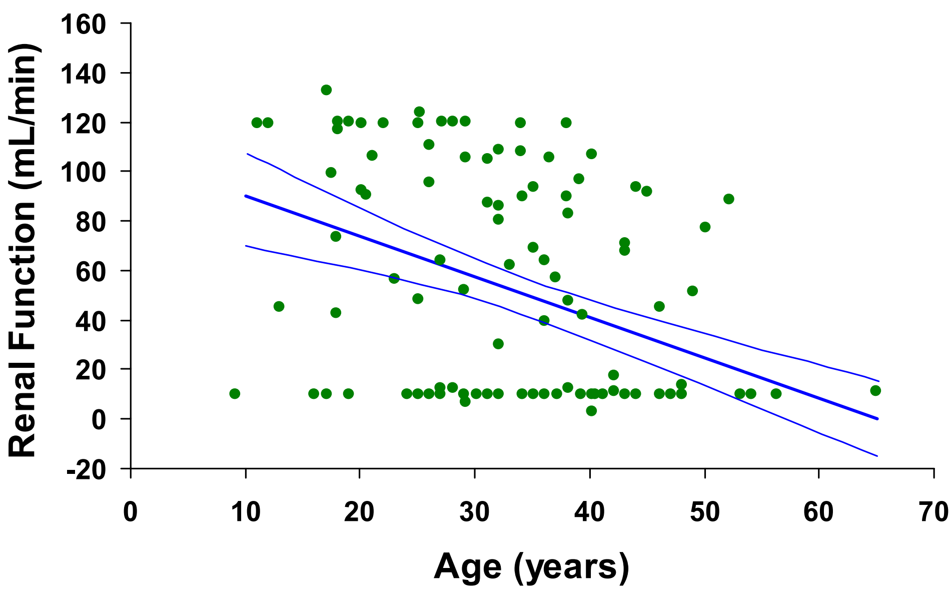
3.2. Kidney Disease

3.2.1. Natural History of Renal Dysfunction

Deterioration of renal function is the most serious complication of Fabry Disease and is the major cause of morbidity and mortality in patients with Fabry Disease. In order to obtain information on the natural history of renal disease, TKT performed a comprehensive review of published English language literature relating to renal function in patients with Fabry Disease. The primary objective of this literature review was to define the natural history of the deterioration of renal function and the progression to ESRD in patients with Fabry Disease. This review identified 116 patients in the literature who had both their age and a measurement of renal function reported.

For these 116 patients the mean age was 33.6±10.4 years and the mean renal function (either creatinine clearance or GFR) was 48.9±44.9 mL/min (normal: ~120 mL/min). Figure 1 below shows a scatterplot of renal function versus age. These data demonstrate that as patients with Fabry Disease age, their renal function deteriorates.

Figure 1: Scatterplot and Linear Regression (with 95% CI) of Renal Function vs. Age (116 patients from the literature)



Since the data presented in Figure 1 do not provide an estimate of the rate of decline of renal function in an individual patient, the literature review also identified individual patients with Fabry Disease who had undergone serial measurements of renal function. These data are summarized in Table 1.

Table 1: Decline of Renal Function over Time

Reference	**Patient Age (from age x→y years)**	**Renal Function Change* (mL/min)**	**Rate of Change (mL/min/yr)**
Ahlmen **[10]**	27 → 29	65 → 52	−6.5
Borok **[11]**	18 → 29	74 → 7	−6.1
Erten **[12]**	25 → 26	50 → 10 (ESRD)	−40
Friedlaender **[13]**	33 → 33.5	62 → 10 (ESRD)	−104
Martinelli **[14]**	36 → 38	40 → 13	−13.5
Philippart **[15]**	32 → 35	30 → 10 (ESRD)	−6.7
Sheth **[16]** (2 patients)	13 → 16 18 → 24	45 → 10 (ESRD) 43 → 10 (ESRD)	−11.7 −5.5
Stiennon **[17]** (3 patients)	38 → 40.5 34 → 36.5 43 → 46	90 → 10 (ESRD) 90 → 106 68 → 46	−32 +6.4 −7.3
Mean **Median**	28.8 → 32.1 yrs		−21 (n=11) −7.3 (n = 11)

*All measurements of renal function are creatinine clearance except for the Ahlmen study that measured renal function with GFR.

These data suggest that the deterioration of renal function can be rapid in patients with Fabry Disease and in some patients is precipitous. The mean decline in the 11 patients in these reports is approximately 21 mL/min/yr.

Branton *et al* recently published a case series of patients from the NIH **[18]**. Among 105 patients followed longitudinally at the NIH, serial measurements of renal function were available for 14 patients. The mean rate of decline of renal function in these patients was 12.2±8.1 mL/min/yr. In addition, Branton *et al* reported that the mean time from the onset of chronic renal insufficiency (defined as a serum creatinine >1.5 mg/dL, which would be equivalent to a creatinine clearance of ~70 mL/min) to ESRD was 4.3 years. The rate of decline of renal function can also be estimated from the decline in renal function in patients randomized to placebo in clinical studies TKT003, TKT005, and TKT010 (see Section 4). Among 59 patients randomized to placebo in these studies the mean rate of decline is 4.13 mL/min over six months, and this can be extrapolated to a rate of decline of 8.3 mL/min/yr. These data are summarized in Table 2.

Table 2: Decline of Renal Function: Summary*

Patient Population	**Patients (Number)**	**Patient Age** (mean)**	**Rate of Decline of Renal Function (mL/min/yr)**
Individual literature patients (Table 1)	11	28.8 → 32.1	21
Branton, *et al*	14	39.8 → 43.1	12.2
TKT003, TKT005, and TKT010 placebo patients	59	35.7 → 36.2	8.3
Mean	84	35.5 → 36.8	10.6

* Renal function in the TKT003, TKT005 and TKT010 placebo patient was measured by GFR, and the individual literature patients' renal function was measured by creatinine clearance, and renal function in the Branton, *et al* study was measured by the MDRD (modification of diet in renal disease) estimate of GFR based on the serum creatinine.

** Mean patient age range over the period of the decline of renal function

The data presented in Table 2 above represent serial measurements of renal function in 84 patients with Fabry Disease. These data suggest that at a mean age of approximately 35.5 years, renal function deteriorates at a rate of approximately 10.6 mL/min/yr. These data are consistent with the mean time from the development of chronic renal insufficiency to ESRD of 4.3 years in patients with Fabry Disease as reported by Branton *et al* [18]. This data also suggests some variability in the rate of decline. Despite this variability, it is clear that Fabry Disease is characterized by a relatively rapid decline in renal function.

The age at onset of ESRD is another way to characterize the natural history of the deterioration of renal function in patients with Fabry Disease. Several case series have examined the progression to ESRD in patients with Fabry Disease and are summarized in Table 3. Each of these studies examined the age at which patients progressed to ESRD.

Table 3: End Stage Renal Disease

References	**Patients (n)**	**Age of Onset of ESRD (years)**
Barnes **[19]**	9	41*
Branton **[18]**	24	39±10
MacDermot **[20]**	26	36.7
Maizel **[21]**	7	43.3
Nissenson **[22]**	17	Median ~40
Ojo **[23]**	93	38±8
Thadhani **[24]**	42	39-42
Tsakiris **[25]**	83	Median 35-44
Individual case reports in the literature†	62	36.7±10.1
Summary	363 patients	~38

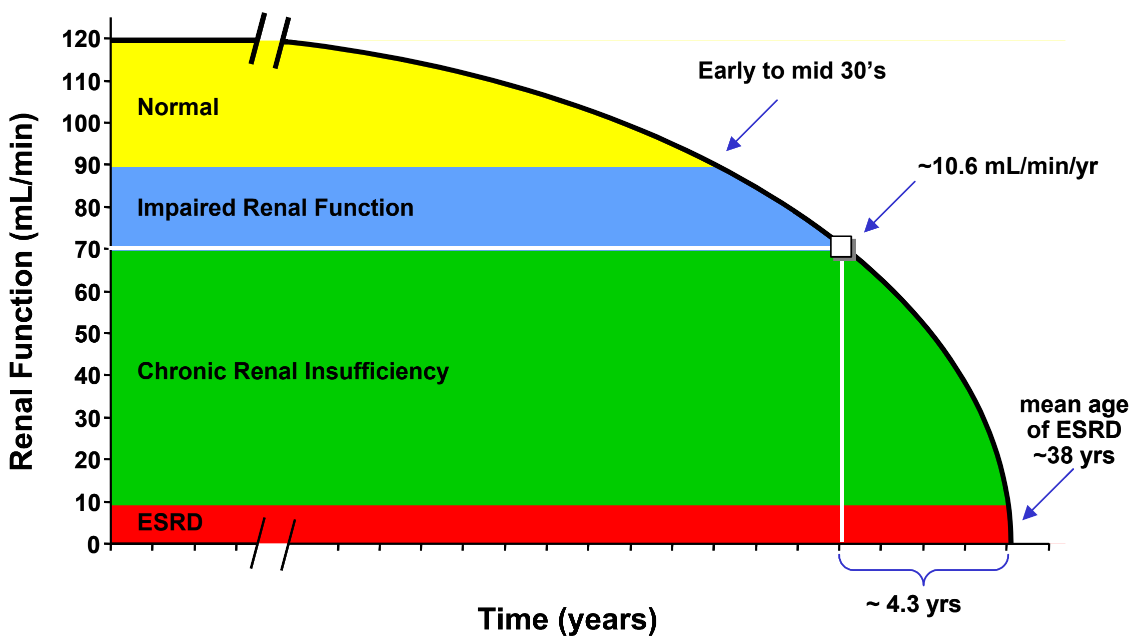
* age at kidney transplant

† 62 individual case reports in the literature.

Based on these data the mean age at which patients with Fabry Disease progress to ESRD has been estimated to be approximately age 38 (see Table 3). To put the renal disease of Fabry Disease in perspective, the mean age of progression to ESRD in the general population is age 62 **[26]**.

Based on the data in the literature, a characteristic curve can be constructed to describe the progression of renal disease in a typical patient with Fabry Disease. A schematic of the drawing of the progression to renal disease in a typical patient is presented in Figure 2.

Figure 2: Fabry Disease Renal Natural History: Summary



In Figure 2, renal function is plotted versus time as measured in years for the typical patient with Fabry Disease. When a patient is younger renal function remains normal due to renal reserve capacity, although there is clearly progressive disease. Most of these patients with renal function in the normal range are less than approximately 30 years old based on the data presented in Figure 1.

However, as a patient's disease progresses, and renal reserve capacity is exhausted, the patient's renal function begins to decline. At a GFR of less than 70 mL/min, a patient has chronic renal insufficiency. Once a patient reaches this level of renal function, a rapid deterioration ensues. The age at which patients are progressing from normal to impaired renal function can be estimated to be in the early 30s. The data presented in Table 2 suggest that the age at which patients begin to deteriorate rapidly is in the mid-30s. Once a patient has impaired renal function, the rate of progression of renal deterioration can be estimated to be approximately 10.6 mL/min/yr. This is consistent with the data presented by Branton *et al* **[18]** that demonstrated a mean time of progression from chronic renal insufficiency to ESRD of approximately 4.3 years. Finally, based on the 363 patients reported in the literature, the mean age at which patients progress to ESRD is approximately age 38 (see Table 3).

In summary, the natural history of renal dysfunction in patients with Fabry Disease is characterized by the following:

- The mean rate of decline of renal function over time is approximately 10.6 mL/min/yr.

- The age at which patients begin to lose renal function at this rate can be estimated to be in the mid-30's.

- The mean age at which patients with Fabry Disease progress to ESRD is approximately age 38.

- The mean time over which patients progress from renal insufficiency to ESRD is approximately 4.3 years. Therefore, the mean age of onset of chronic renal insufficiency occurs at approximately age 34 (38 years minus approximately 4.3 years).

- The natural history of Fabry Disease is characterized by a deterioration of renal function beginning in the mid-30's leading to ESRD in the late 30's.

3.2.2. Renal Pathology of Fabry Disease

3.2.2.1. Fabry Disease: Involvement of the Epithelium and Mesangium

The two major renal cell types affected in Fabry disease are the glomerular epithelial cells (also known as podocytes) and the glomerular mesangial cells. Their structure and function will be discussed briefly. In addition, renal tubular epithelial cells and renal blood vessels are involved to a lesser extent.

Glomerular epithelial cells (podocytes) are the largest cells in the glomerulus. Elongated foot processes from the podocytes come into contact with the external surface of the basement membrane. The podocytes synthesize materials that play an active role in the biogenesis of the basement membrane. The basement membrane is critical for the selective sieving of proteins. Accordingly, podocyte damage leads to proteinuria. The end result of chronic podocyte damage is glomerular obsolescence and concomitant renal failure.

Mesangial cells help maintain glomerular architecture, and provide support for the network of capillaries that enter the glomerulus. Mesangial widening and increased matrix synthesis contributes to the reduction of the surface available for filtration by promoting glomerular sclerosis. The consequence of chronic mesangial widening is also glomerular obsolescence and concomitant renal failure.

Fabry Disease is also characterized by focal glomerular sclerosis. The mechanism for this lesion likely includes epithelial cell injury, retraction of the foot processes, areas of capillary cells that are devoid of foot processes cover, and subsequent segmental capillary collapse. This leads to increased convective flow past the glomerulus, with resultant proteinuria. Mesangial expansion is another critical piece in the pathogenesis of focal sclerosis, and it is thought that trophic and growth factors released from platelets and inflammatory cells influence the proliferation of mesangial cells and modify their rate of matrix and scar formation, all of which lead to reduced capillary surface area for filtration.

The hallmark of renal pathology of Fabry Disease is that functional glomeruli are gradually replaced by dysfunctional cells with significant Gb_3 deposition. The two major renal cell types that are affected in Fabry Disease are the glomerular epithelial cells (podocytes) and glomerular mesangial cells.

The pathology of Fabry Disease of the kidney has been well described:

From Dr. Barry Brenner's textbook entitled 'Brenner & Rector's The Kidney' **[27]**:

> "***Glycosphingolipid accumulation begins early in life, and the major site of accumulation is the glomerular epithelial cell*** [emphasis added] (visceral cells more so than parietal cells). By LM, these cells are enlarged with numerous clear, uniform vacuoles in the cytoplasm, causing a foamy appearance. These vacuoles can be shown to contain lipids when fat stains (such as oil red O) are used, or when they are viewed under the polarizing microscope where they exhibit a double refractile appearance before being processed with lipid-soluble stains. All renal cells may accumulate the lipid, including glomerular endothelial cells and mesangial cells. Tubular epithelium also shows involvement with distal convoluted tubules and loops of Henle being more severely involved than proximal tubular cells. Myocytes and endothelial cells of arteries also may show similar changes."

From Dr. Robert Desnick's chapter in: The Metabolic & Molecular Bases of Inherited Disease **[2]**

> "The lesions are due to the accumulation of glycosphingolipids ***primarily in epithelial cells of the glomerulus and of the distal tubules*** [emphasis added]. In later stages, and to a lesser degree, proximal tubules, interstitial histiocytes, and interstitial cells may show lipid accumulation. Lipid-laden distal tubular epithelial cells desquamate and may be detected in the urinary sediment. These cells have been shown to account for about 75 percent of the urinary cells shed by an affected hemizygote."

> "Concurrently, renal blood vessels are involved progressively and often extensively. A late finding is arterial fibrinoid deposits, which may result from the necrosis of severely involved muscular cells. Other histologic changes in the kidney are the sequelae arteriolar sclerosis, glomerular atrophy and fibrosis, tubular atrophy, diffuse interstitial fibrosis, and other secondary changes. Renal size increases during the third decade of life, followed by a decrease in the fourth and fifth decades. The renal involvement has been the subject of a recent report and older comprehensive reviews."

Glomerular capillary endothelial cells, as well as endothelial cells throughout the kidney, are relatively spared **[2,29]**. As described above, vascular involvement in the kidney principally involves the large vessels. Gb_3 deposition primarily affects the muscular cells of arterioles and the large arteries.

Following the initial insult of Gb_3 deposition in the glomerular epithelial cells and mesangial cells, there is initial expansion of the mesangial matrix leading to mesangial widening. Subsequently, the expansion of the mesangial matrix leads to scarring manifested by focal segmental glomerulosclerosis. Ultimately, the pathologic progression of disease in the kidney culminates with glomerular obsolescence signaling the loss of that individual nephron.

3.2.2.2. Fabry Disease: Involvement of the Endothelium

In contrast to Fabry Disease, there are specific renal diseases that primarily involve injury to capillary endothelial cells and that lead to renal failure. Clinically, these diseases differ markedly from Fabry Disease. The features of endothelial cell diseases of the kidney are summarized in Table 4.

Table 4: Endothelial Cell Diseases of the Kidney

Disease	Putative Mechanism of Injury	Light Microscope Findings	Clinical Findings
Preeclampsia	Direct Injury	Occlusion of the capillary lumen	Proteinuria
Membranoproliferative Glomerulonephritis	Immune Complex Injury	Capillary wall thickening, reduplication of the basement membranes	Heavy proteinuria, Hematuria
Thrombotic Microangiopathies	Direct Injury	Swelling of endothelial cells, occlusion of capillary lumens, reduplication of the basement membrane	Hematuria, Proteinuria

Injury to the capillary wall of the glomerulus results from immune complex deposition in some diseases, while other diseases appear to be caused by direct injury to the endothelial cell. In the setting of immune deposits, inflammatory cells with evidence of capillary wall destruction are evident, and clinically, hematuria is classically seen. In non-immune causes of endothelial cell injury, platelet aggregation and fibrin debris are often present, and an active urinary sediment with hematuria is seen. In many causes of endothelial cell injury in the glomerulus, the glomerular wall undergoes repeated injury, and the glomerular capillary wall thickens and can duplicate, resulting in double contours seen on light and electron microscopy. Other features of direct endothelial cell injury include swelling of the capillary endothelial cytoplasm and occlusion of the capillary lumens. Finally, chronic ischemia of the glomerulus results in wrinkling of the basement membrane.

Fabry disease is not known to be associated with duplication or thickening of the basement membrane, wrinkling of the basement membrane is not seen, and the capillary lumens are open without evidence of occlusion. Hence, endothelial cell involvement may be evident, but it is unlikely to cause glomerular pathology and renal failure by itself. In addition, the clinical features of Fabry Disease (see Table 5) are markedly different from the endothelial cell diseases shown in Table 4.

3.2.2.3. Fabry Disease: Histopathological Findings

The histology of the normal kidney is shown in Figure 3. The glomerular architecture is normal with a delicate basement membrane, patent capillaries, and normal mesangial matrix and cellularity. There is no evidence of glycosphingolipid deposition. The renal pathology of Fabry Disease is shown in Figures 4 and 5. Figure 4 displays the epithelial nature of the glomerular pathology of Fabry Disease. Figure 4A is a photomicrograph of a typically involved glomerulus in a patient with Fabry Disease. The section is stained with toluidine blue, which highlights the Gb_3 deposits as bright blue round inclusion bodies within the epithelial cells. The renal epithelial cells and mesangial cells are swollen with Gb_3 deposits with early evidence of mesangial widening (see below). In contrast, clear and patent capillary loops are easily seen in this photomicrograph (capillaries are identified by the presence of the red blood cells, with capillary endothelial cells that are free of Gb_3 deposits).

Figure 4B is a higher power magnification of Figure 4A. In this view the predominance of epithelial involvement is clearly seen. There are marked Gb_3 depositions within the podocytes, but capillary endothelial cells are essentially free of deposition. As discussed above, the pathologic hallmarks of endothelial cell disease in the kidney (double contours, swelling of the capillary endothelial, cytoplasm and, and occlusion of capillary lumens) are not seen in Fabry Disease. In addition, the pathologic consequence of chronic ischemia (wrinkling of the basement membrane) is also not a component of the kidney pathology of Fabry Disease.

Figure 4C is an electron micrograph of a glomerulus in a patient with Fabry Disease. The electron-dense deposits of Gb_3 (seen as dark multilamellar or Zebra bodies in the electronmicrograph) are clearly seen within swollen podocytes. Again, the capillary loops, identified by the presence of the densely staining red blood cells, are free of deposition and the capillary endothelial cells are essentially normal.

Figure 3: Normal Glomerulus (PAS Stain)

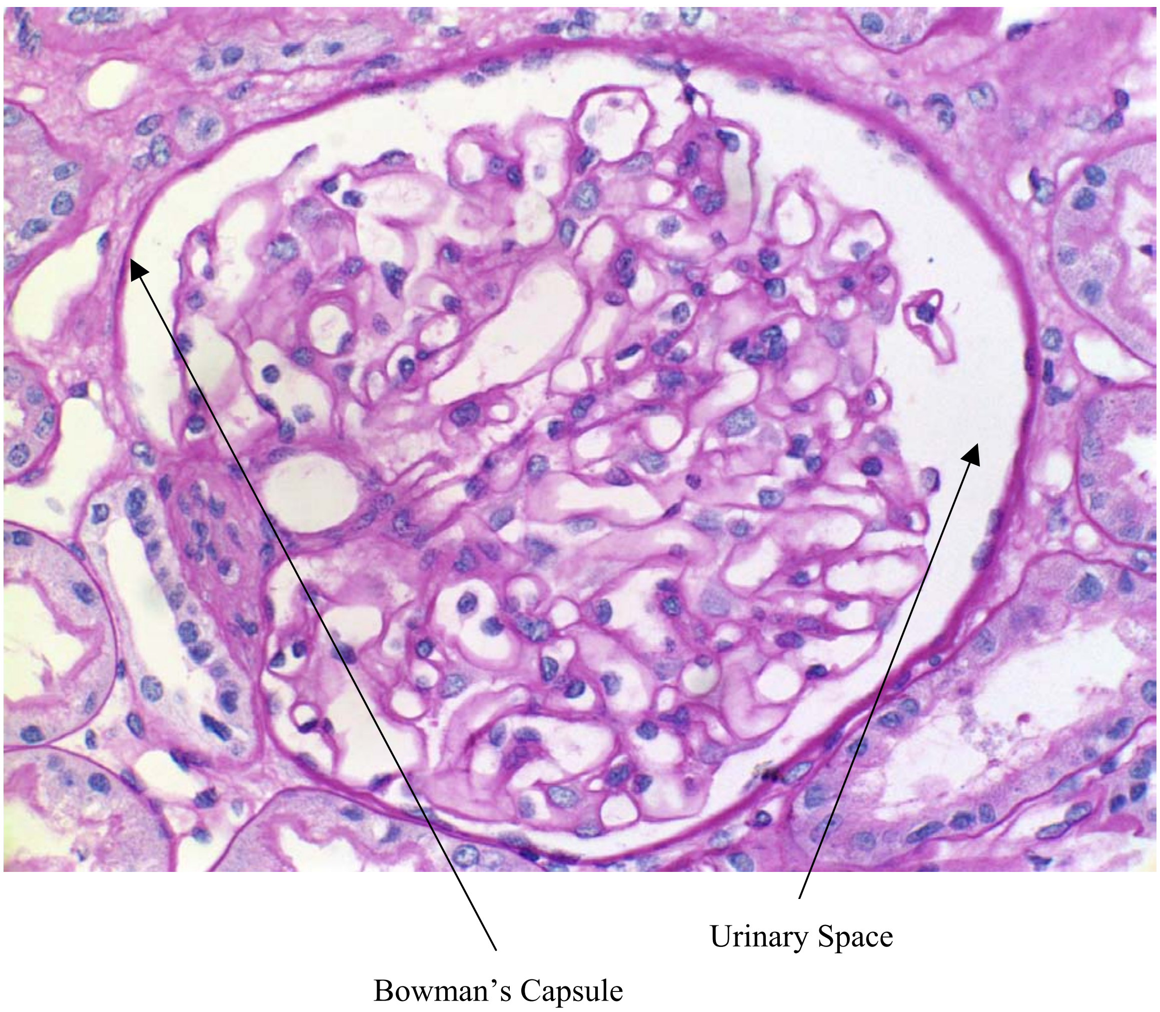


Figure 4A: Fabry Glomerulus (Toluidine Blue Stain; 40x)

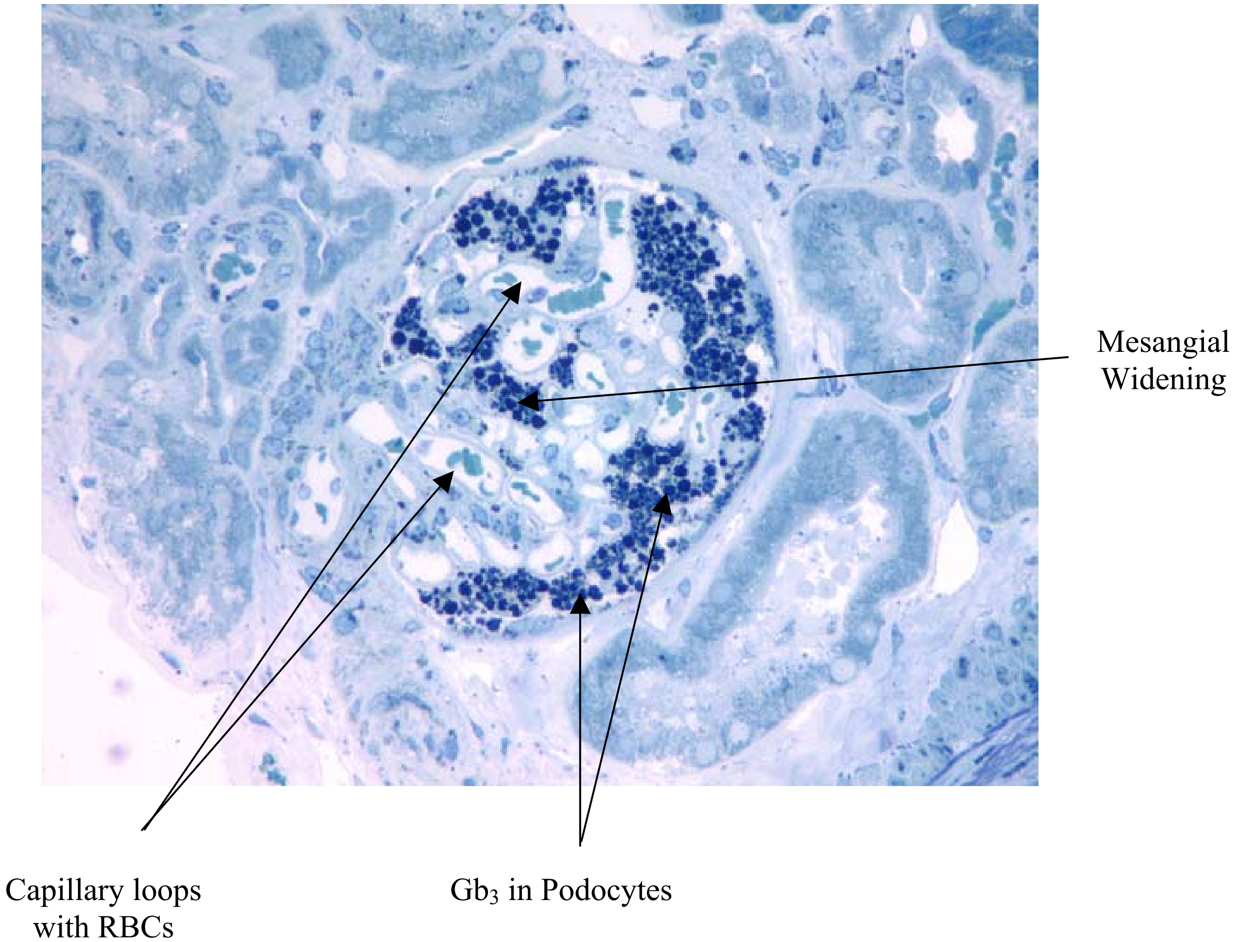


Figure 4B: Fabry Glomerulus at High Power (Toluidine Blue Stain; 100x)

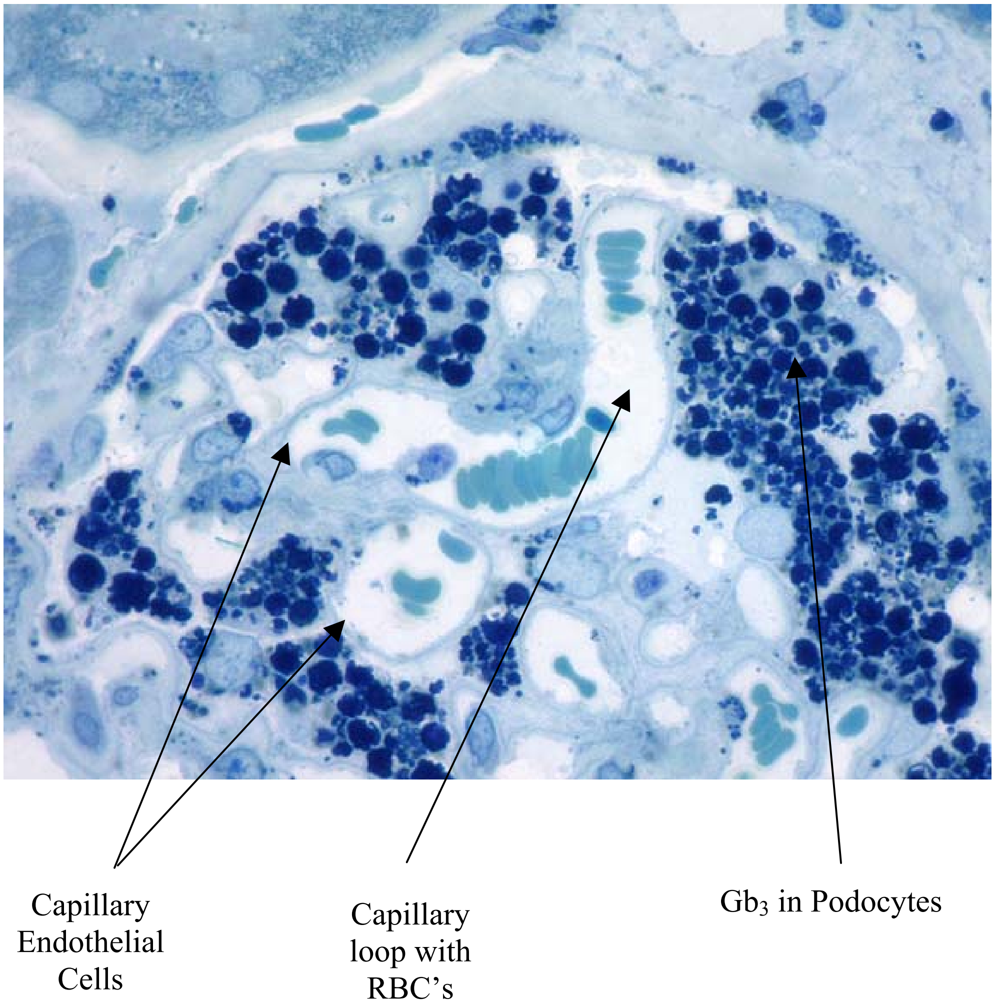
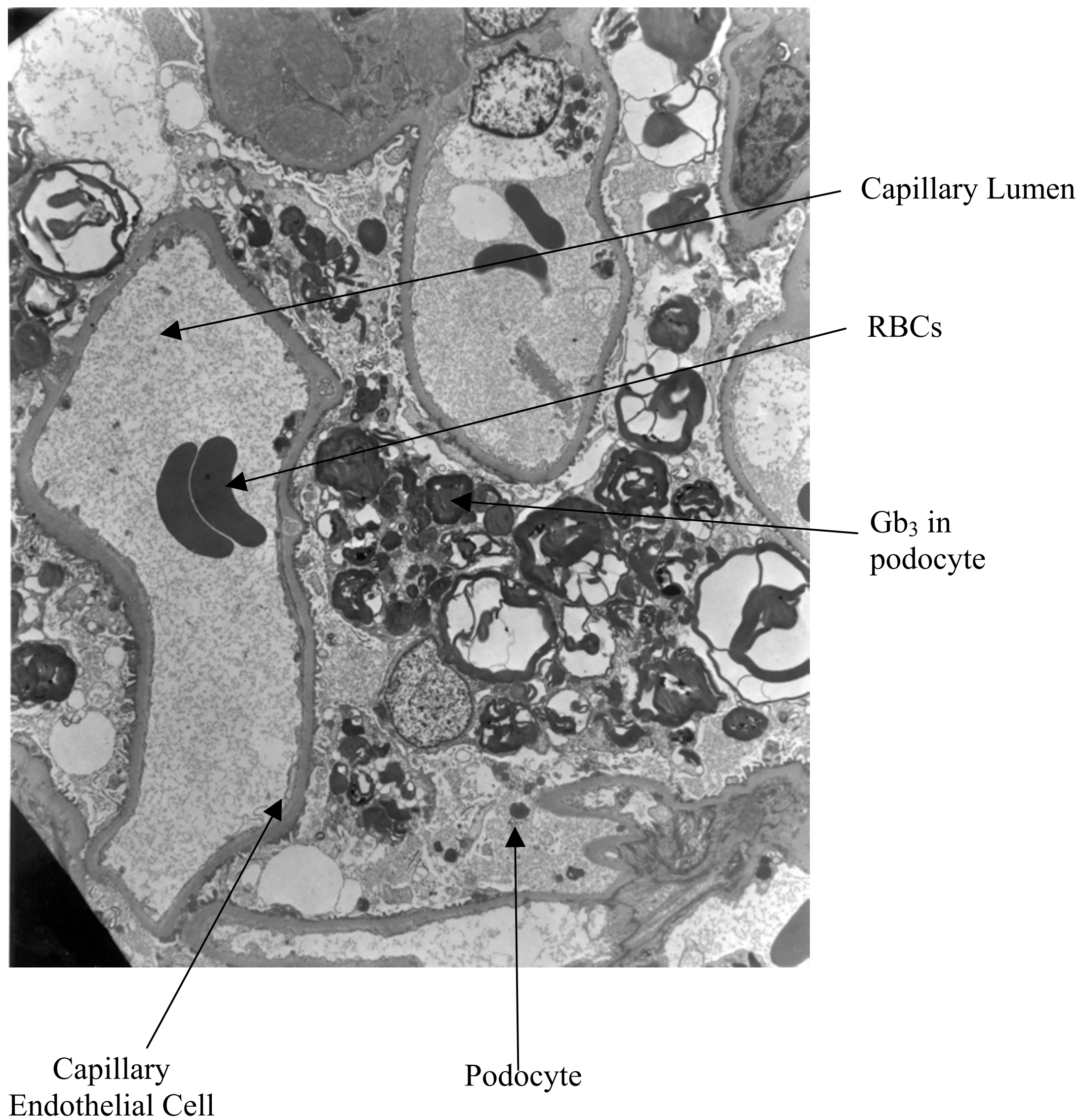


Figure 4C: Fabry Glomerulus: Electron Microscopy



Once Gb_3 has damaged the podocytes and mesangial cells, a pathologic spectrum of disease is seen, ranging from essentially normal glomeruli to sclerotic and obsolescent glomeruli. Representative examples of the spectrum of progression from normal architecture to glomerular obsolescence are shown in Figures 5A through 5D. Each figure contains a PAS and toluidine blue stained section. Although Gb_3 deposition is an early finding in Fabry glomeruli, essentially normal glomerular architecture is present. Glomeruli with essentially normal architecture (see Figure 5A) are followed by glomeruli with mesangial widening (see Figure 5B). Subsequently, mesangial widening can progress to focal and segmental glomerular sclerosis (see Figure 5C). This is followed by glomerular obsolescence (see Figure 5D). The initial pathological insult (Gb_3 deposition) is much less apparent in end-stage (sclerotic and obsolescent) glomeruli. Notably, the obsolescent glomerulus of Figure 5D-2 shows very little staining with toluidine blue, as the glomerulus has undergone fibrosis. In general, as kidney pathology progresses there is evidence of decreased Gb_3 storage as lipid-laden epithelial and mesangial cells are replaced by scar tissue. In addition, there is evidence of histopathological worsening characterized by focal and segmental glomerulosclerosis and overt glomerular obsolescence.

Figure 5A-1: Fabry Glomerulus: Essentially Normal Architecture (PAS Stain)

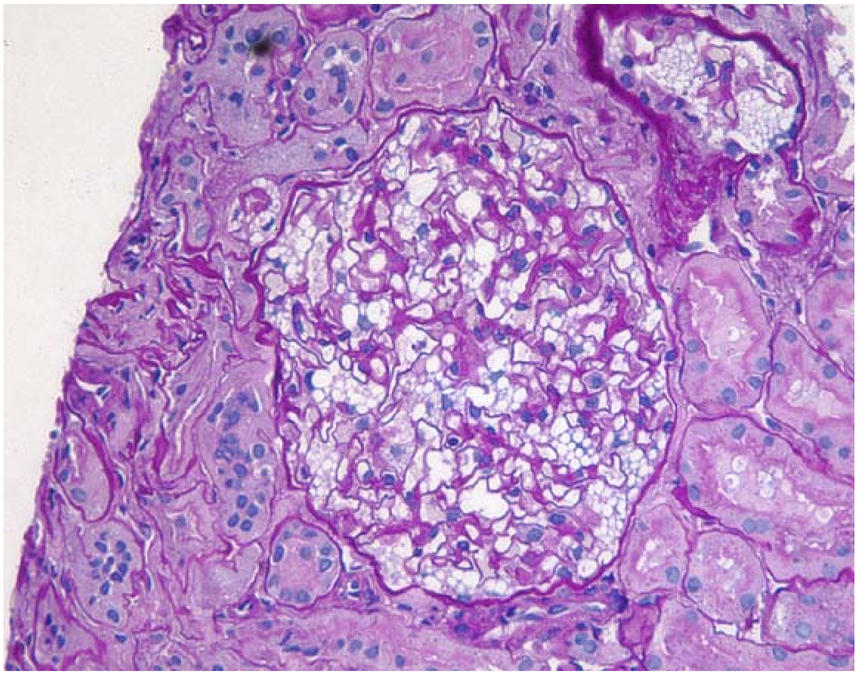


Figure 5A-2: Fabry Glomerulus: Essentially Normal Architecture (Toluidine Blue)

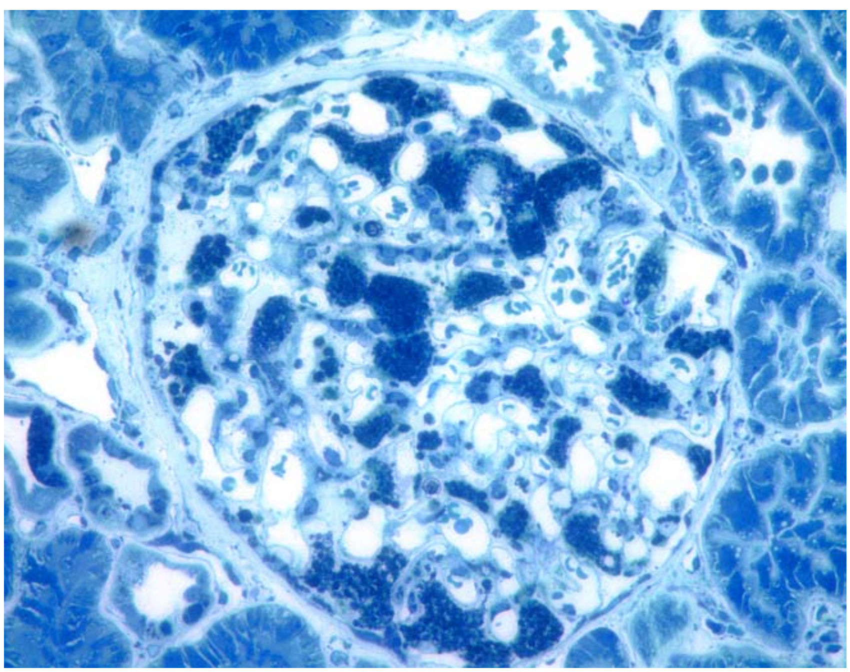


Figure 5B-1: Fabry Glomerulus: Mesangial Widening (PAS Stain; 40x)

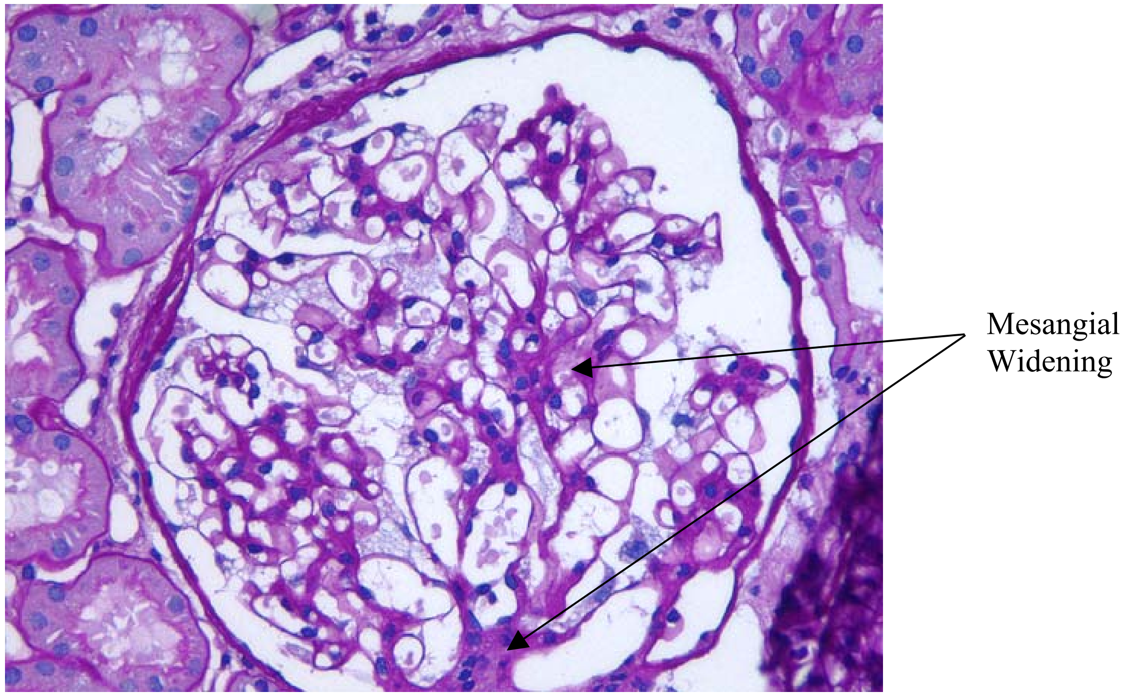


Figure 5B-2: Fabry Glomerulus: Mesangial Widening (Toluidine Blue Stain; 40x)

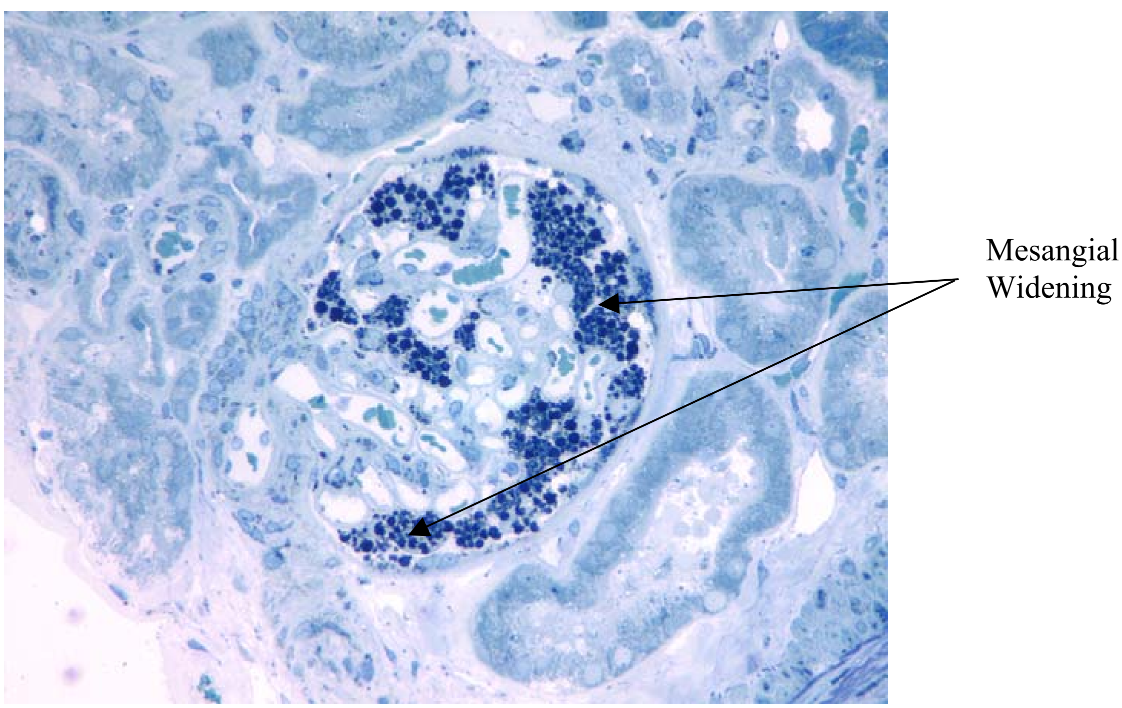
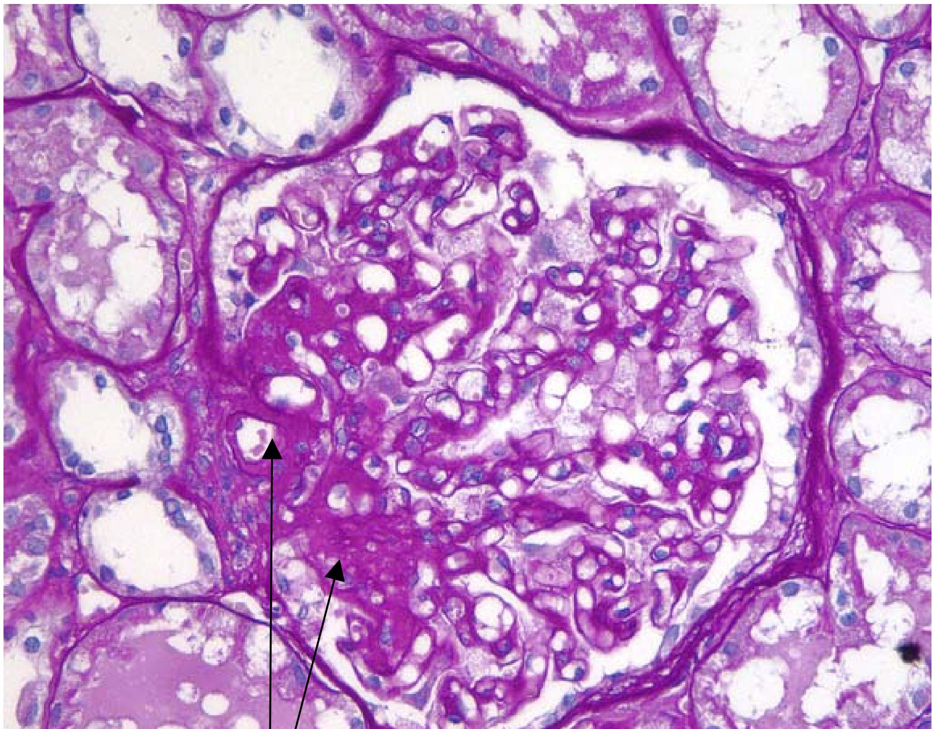


Figure 5C-1: Fabry Glomerulus: Segmental Sclerosis (PAS Stain; 40x)



Segmental Sclerosis

Figure 5C-2: Fabry Glomerulus: Segmental Sclerosis (Toluidine Blue Stain; 40x)

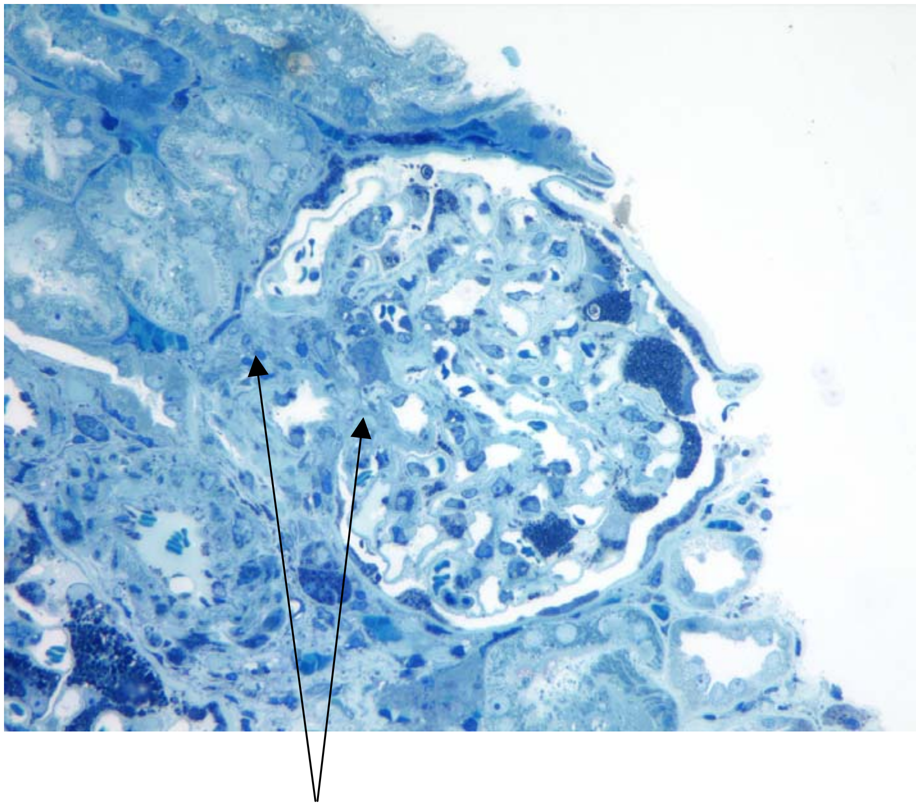


Table 5D-1: Fabry Glomerulus: Obsolescence (PAS Stain; 40x)

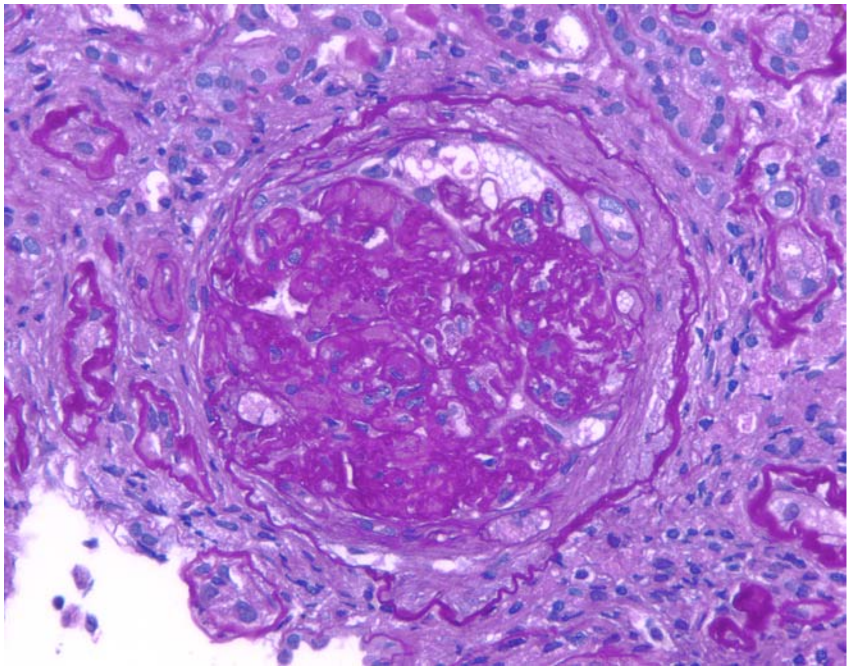
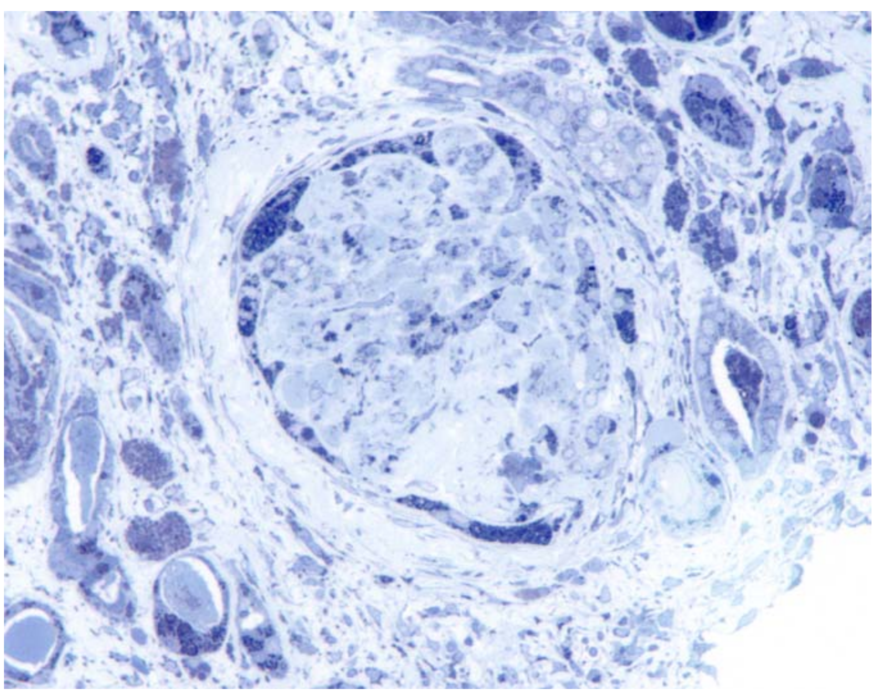


Figure 5D-2: Fabry Glomerulus: Obsolesence (Toluidine Blue Stain; 40x)



In summary, the glomerular pathology of Fabry Disease is initiated by Gb_3 deposition in glomerular epithelial cells (podocytes) and mesangial cells. This damage eventually leads to glomeruli with mesangial widening and focal sclerosis, and culminates with obsolescence of the glomeruli.

3.2.3. Clinicopathological Correlation – Renal Disease

As discussed above, there is a progression of pathologic changes in the kidneys of patients with Fabry Disease. This progression of pathological changes correlates with the clinical progression of Fabry Disease. Table 5 summarizes the correlation of the progression of pathology changes with the progression of clinical disease in the kidney.

Table 5: Fabry Disease: Clinicopathological Correlation in the Kidney

Stage of Disease	Pathology	Clinical Correlates
Early	**Glomeruli** • Vacuolization of epithelial cells • Mesangial widening **Tubules** • Mild damage	**Glomeruli** • Proteinuria • Impaired renal function (decreased glomerular filtration rate) **Tubules** • Renal concentrating defects (isosthenuria)
Late	**Glomeruli** • Segmental sclerosis • Glomerular obsolescence **Tubules** • Diffuse tubular epithelial cell damage	**Glomeruli** • Nephrotic syndrome • Chronic renal insufficiency (GFR less than 70 mL/min) • ESRD **Tubules** • Severe concentrating defects, including diabetes insipidus

One of the earliest clinical manifestations of renal dysfunction is proteinuria - a consequence of podocyte injury. Proteinuria is a well established clinical consequence of glomerular epithelial cell damage. In Fabry Disease, proteinuria is one of the earliest clinical signs of renal dysfunction. Gubler *et al* described nine young male patients with newly diagnosed Fabry Disease **[28]**. These patients had early pathologic changes in the kidney, including podocyte injury, and four patients had measurable proteinuria. Proteinuria is an established independent risk factor for the progression of renal disease. Initially, as with these patients, proteinuria is often not associated with decreased GFR **[29]**. As podocyte damage and mesangial widening progress, renal reserve capacity is exhausted and renal function begins to decline.

There are two major late clinical manifestations of Fabry Disease in the kidney, and these are explained by the late pathologic changes. First, there is a progressive loss of glomerular filtration rate that is manifested as chronic renal insufficiency, which ultimately culminates in ESRD. The progressive renal dysfunction reflects the progressive loss of functional glomeruli and their subsequent replacement by sclerotic and obsolescent glomeruli. Secondly, the hyposthenuria and vasopressin-resistant diabetes insipidus reflect the additional damage to the collecting system and the loss of concentrating ability.

3.3. Heart Disease

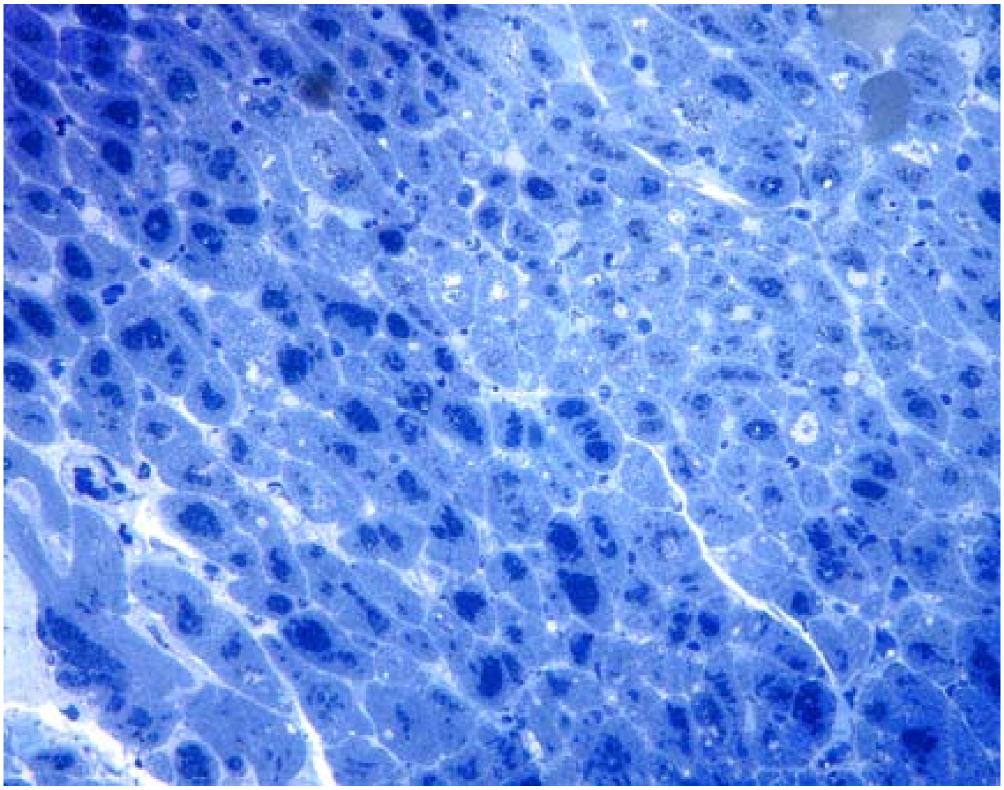
In the heart, both cardiac myocytes and valvular fibrocytes accumulate Gb_3. A wide variety of cardiac signs and symptoms, together with a correspondingly diverse spectrum of pathological changes characterize the cardiomyopathy of Fabry Disease. Clinically, this cardiomyopathy is characterized by angina pectoris, myocardial ischemia, arrhythmias, myocardial infarction, congestive heart failure, and aortic and mitral valve dysfunction. Table 6 summarizes the cardiac clinicopathological correlations in Fabry Disease.

Table 6: Fabry Disease: Clinicopathological Correlation in the Heart

Stage of Disease	Pathology	Clinical Correlates
Early	• Patchy vacuolization of myocytes with lamellar inclusion bodies • Mitral valve damage	• Abnormal electrocardiogram, mild left ventricular hypertrophy • Mitral valve prolapse and regurgitation
Late	• Foamy vacuolization of myocytes • Cellular hypertrophy • Left ventricular free wall thickening • Interventricular septal thickening • Cor bovinum	• Cardiomegaly • Severe left ventricular hypertrophy • Prolonged QRS complex and bundle branch blocks • End stage cardiac disease and death

Figure 6 is a photomicrograph of the heart of a patient with Fabry Disease. The photomicrograph is stained with toluidine blue, which highlights Gb_3 deposits as dark blue circular inclusions. Deposition within essentially all cardiac myocytes is seen. As seen in Figure 6, there is extensive cellular hypertrophy. In addition, there is distortion of the normal myocyte architecture with myocyte disarray. The myocyte disarray likely contributes to the cellular hypertrophy that is responsible for the pathophysiology of LVH in these patients. As is the case with the kidney, the endothelial capillary cells are relatively free of Gb_3 deposits in the heart.

Figure 6: Fabry Disease: Heart Biopsy (Toluidine Blue Stain; 40x)



Fabry Disease is an intracellular deposition cardiomyopathy. The cardiomyopathy of Fabry Disease is a progressive and hypertrophic cardiomyopathy. Several studies have demonstrated a progressive age-related increase in cardiac mass, consistent with the progression of cardiomyopathy, in both male and female patients with Fabry Disease. **[30**,**31**,**32**,**33]**. Goldman *et al* demonstrated a significant difference in cardiac mass between males older than age 25 and males younger than age 25. Linhart has demonstrated a significant correlation of age with the degree of LVH. Finally, Kampmann has also demonstrated a significant and progressive increase in LVH in female patients with Fabry Disease.

Approximately 20% of patients with Fabry Disease die of cardiomyopathy-related causes.

3.4. Other Aspects of Fabry Disease

3.4.1. Nervous System

3.4.1.1. Pain

Chronic pain has been described as the most debilitating symptom in patients with Fabry Disease. Pain can be so difficult to control that suicide has been described in Fabry patients as a result.

There are two different pain syndromes for patients with Fabry Disease. The majority of patients suffer from a constant burning pain in their hands and feet. This pain is accompanied by paresthesias (numbness and constant tingling in the hands and feet). These "acroparesthesias" are symptomatically challenging. Most patients require therapy with anticonvulsants such as diphenylhydantoin (Dilantin®) or carbamazepine (Tegretol®), consistent with the neuropathic aspect of this type of pain.

A second syndrome usually begins in childhood, and is characterized by episodic, severe crises of pain in the extremities and abdomen. These crises are characterized by lancinating pain that shoots centrally from the hands and feet. Fever and joint pains accompany such crises. These crises, as well as the chronic pain in the extremities, are a source of major morbidity in Fabry Disease.

3.4.1.2. Hearing

Patients with Fabry Disease suffer from sensorineural hearing loss. MacDermot *et al* described high frequency sensorineural hearing loss that can be either bilateral or unilateral in patients with Fabry Disease **[20]**. This is often under-reported by patients, as MacDermot *et al* found that 41% of patients reported hearing loss, but 78% of patients had an abnormal audiogram.

3.4.2. Gastrointestinal Disease

Gastrointestinal involvement, with associated diarrhea and abdominal pain, is an important cause of morbidity in patients with Fabry Disease, and is probably also under-reported **[2, 34]**. Case reports reveal a diverse set of gastrointestinal syndromes including anorexia, diarrhea, achalasia, and jejunal diverticulosis with perforation, and the most common result of gastrointestinal disease is chronic weight loss **[35,36,37]**.

A comprehensive evaluation of gastrointestinal disease in patients with Fabry Disease was performed by Sheth *et al* **[38]**. Table 7 summarizes the gastrointestinal symptoms in their cohort of patients. Two-thirds of the patients studied had gastrointestinal symptoms.

Table 7: Gastrointestinal Symptoms in Fabry Disease

Symptom	Percent of Patients
Chronic Diarrhea	24
Recurrent Abdominal Pain	20
Diarrhea/Constipation	12
Postprandial Cramping	8
Early Satiety	4
Any Symptom	68

3.4.3. Cerebrovascular Disease

A relatively late finding in patients with Fabry Disease is cerebrovascular disease. MacDermot *et al* reported an incidence of stroke or transient ischemic attacks of 24% in 70 male patients studied **[20].** The mean age of onset of stroke was 40.4 years. In addition, Moore *et al* have recently characterized cerebrovascular blood flow abnormalities in patients with Fabry Disease **[39]**. They demonstrated that patients with

Fabry Disease have abnormally elevated cerebrovascular blood flow and speculated that these cerebrovascular blood flow abnormalities may be related to the pathophysiology of stroke in these patients.

3.5. Clinicopathological Overview of Fabry Disease: Conclusions

Fabry Disease is a complex, multisystem clinical syndrome caused by a deficiency of the lysosomal enzyme α-galactosidase A. Throughout the course of the disease, there is a gradual and progressive pathologic deposition of Gb_3 in the parenchymal cells of the kidney (glomerular epithelial cells or podocytes, mesangial cells, and tubular epithelial cells) and heart (cardiac myocytes), and the subsequent pathological and the major clinical sequelae include progressive renal and cardiac failure.

4. Clinical Experience with Replagal in Fabry Disease

4.1. Overview

The clinical development program for Replagal included clinical endpoints assessing the major organ systems most severely affected in Fabry Disease. The program also explored the effects of Replagal on other aspects of Fabry Disease including the effect on body weight, cerebrovascular blood flow, and hearing. Following discussions with the FDA regarding difficulties in interpretation of the pain aspects of TKT's clinical development program for Replagal, this briefing book does not include any discussion of the clinical data describing the effects of Replagal on the pain of Fabry Disease.

Studies with Replagal have been conducted at three clinical sites: the United States National Institutes of Health (NIH), the Royal Free Hospital (RFH) in London, United Kingdom, and the University of Mainz in Germany. An overview of the clinical studies submitted to the BLA is presented in Table 8 below. In addition, the Replagal safety database now includes information on over 300 patients, including those on commercial Replagal therapy in Europe.

Table 8: Clinical Studies Submitted with the Replagal BLA

Protocol	Design	# Pts	Duration
NIH Clinical Studies			
TKT001	Open label, dose escalation safety study	10	Single dose
TKT003	Randomized, double blind, placebo controlled Primary endpoint: pain Additional endpoints: renal pathology and function	**26**	6 months
TKT006	Open label maintenance study for patients completing TKT003 Primary endpoints: cardiac mass, GFR and pain	25	1 year
TKT011	Open label maintenance study for patients completing TKT006 Primary endpoint: home safety Additional endpoints: sentinel clinical events (eg, renal function)	24	1 year analysis (Study Ongoing)
RFH Clinical Study			
TKT005	Randomized, double blind, placebo controlled Primary endpoint: cardiac Gb_3 Additional endpoints: Cardiac Mass (MRI and Echo)	**15**	6 months
University of Mainz Clinical Study			
TKT014	Open label safety and efficacy trial in female patients Primary endpoint: urine sediment Gb_3 Additional endpoints: Cardiac mass and function (Echo)	**15**	3-12 months
TOTAL:	Multidose Studies	**56***	>2.5 years

* Total number of unique patients in multidose clinical studies of Replagal (26 patients from Study TKT003, 15 patients from Study TKT005, and 15 patients in Study TKT014).

Note: Preliminary data from a third double blind, placebo-controlled study have recently been obtained by TKT. This study, TKT010, was only unblinded in November of 2002; therefore, the complete data set has not been fully analyzed, and a study report has not yet been submitted to the BLA. However, preliminary data for the primary endpoint of glomerular filtration rate (GFR) is included in this briefing book for completeness.

4.1.1. NIH Studies

Study TKT001 was a dose escalation study. This study generated the single dose safety profile, pharmacokinetic and biodistribution data, and dose-response information demonstrating that with increasing doses of enzyme, the liver takes up a progressively smaller fraction of the dose. Data from this study were used, in part, to establish the clinical dose of Replagal used in all subsequent clinical studies.

Study TKT003 was a randomized, double blind, placebo controlled study conducted over six months. Patients were randomized to receive either 0.2 mg/kg of Replagal or placebo on an every other week basis. The primary endpoint was an assessment of serious, debilitating pain as measured by the Brief Pain Inventory (BPI). Additional efficacy endpoints included assessments of kidney function and kidney pathology.

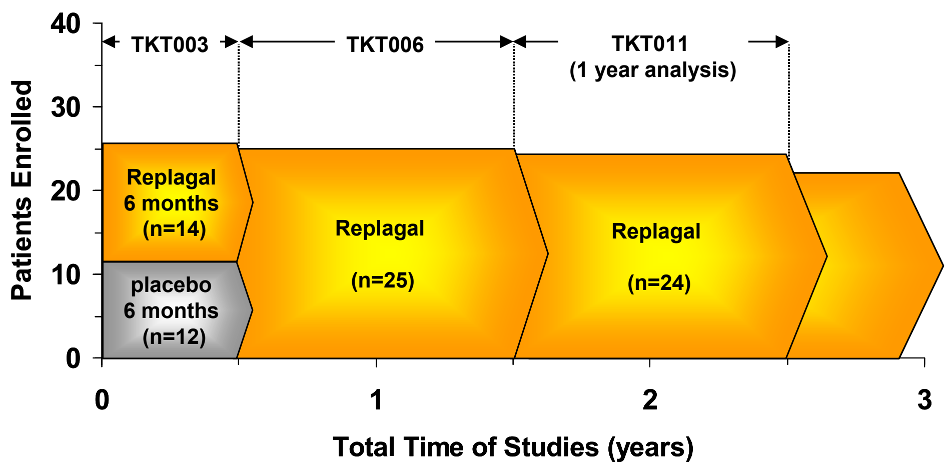
Study TKT006 was an open label long-term maintenance study conducted over one year for patients who completed Study TKT003. The primary efficacy analyses were the effect of Replagal on the change from baseline to Week 52 for: (1) cardiac mass as determined by MRI, (2) renal function as determined by GFR, and (3) pain as determined by the BPI. Additional efficacy endpoints included measurements of creatinine clearance.

Study TKT011 is an open label long-term maintenance study for patients who completed Study TKT006. The primary objective of this study is to evaluate the long-term safety of Replagal. Additional efficacy endpoints include the incidence of sentinel clinical events (including the initiation of renal dialysis) and the long-term effect of Replagal on renal function as measured by GFR and creatinine clearance. This study is also designed to assess the safety of home infusions of Replagal.

Patients in Study TKT011 continue to receive Replagal. The patients initially randomized to Replagal in Study TKT003 have received Replagal for 2.5 years (six months in TKT003, one year in TKT006, and one year in TKT011), and the patients initially randomized to placebo in Study TKT003 have received Replagal for two years (one year in TKT006 and one year in TKT011). Study TKT011 is ongoing.

A schematic representation of the relationship between studies TKT003, TKT006, and TKT011 is presented in Figure 7 (below).

Figure 7: NIH Clinical Studies TKT003, TKT006, and TKT011: 2.5 Year Summary



4.1.2. RFH Study

Study TKT005 was a randomized, double blind, placebo controlled trial conducted over six months. Patients were randomized to receive either 0.2 mg/kg of Replagal or placebo on an every other week basis. Patients were included in this study based on echocardiographically determined evidence of left ventricular hypertrophy. The primary efficacy endpoint was the measurement of Gb_3 content in endomyocardial biopsy specimens. Additional efficacy endpoints included an assessment of the effect of enzyme replacement therapy on cardiac mass as determined by MRI and Echo. Safety was determined by standard clinical and laboratory measurements.

4.1.3. University of Mainz Study

Study TKT014 was an open label study of the safety and efficacy of Replagal in female patients with Fabry Disease. The primary clinical efficacy endpoint was an effect of Replagal therapy on urine sediment Gb_3 levels. Additional efficacy endpoints included measurements of the effect of Replagal therapy on cardiac mass and cardiac function as measured by echocardiography. Safety was determined by standard clinical and laboratory measurements.

4.1.4. Study TKT010

Study TKT010 was a randomized, double blind, placebo controlled study conducted at 10 sites world-wide. 80 patients were enrolled in the study, with 40 patients randomized to Replagal and 40 patients randomized to placebo. Study TKT010 was conducted over six months. The primary efficacy endpoint was the assessment of renal function as measured by GFR. Safety was determined by standard clinical and laboratory measurements. Analysis of the data is currently in progress.

4.2. Summary of Efficacy Results

Six clinical studies (TKT003, TKT006, TKT011 at the NIH, TKT005 at RFH, TKT014 at the University of Mainz, and Study TKT010) described above have provided efficacy data regarding the effects of Replagal on renal function, cardiomyopathy, and metabolism in patients with Fabry Disease. Data from these studies demonstrate the following:

Renal Effects

- Study TKT003 demonstrated that patients who received Replagal had stable renal function compared to a decline in renal function in patients who received placebo for six months. However, Study TKT010 did not demonstrate a difference in renal function between patients randomized to placebo and patients randomized to Replagal after six months.

- Long term therapy with Replagal results in improvements in renal function. Data from Study TKT011 demonstrate that Replagal stabilizes renal function and over the course of 2-2.5 years improves renal function.

- Replagal delays and may prevent the progression to ESRD for patients with Fabry Disease. Based on TKT's literature study of the natural history of Fabry Disease, the mean age at which patients progress to ESRD is approximately 38 years. Patients treated with Replagal in Studies TKT003, TKT006, and TKT011 are currently approximately 38 years old. No patient who has received Replagal has progressed to ESRD, and renal function has been stabilized in these patients.

- Replagal improves the underlying renal pathology of Fabry Disease compared with placebo. Replagal increased the fraction of normal glomeruli compared with a decrease in the fraction of normal glomeruli in patients treated with placebo. In addition, Replagal decreased the fraction of glomeruli with mesangial widening compared to an increase in the fraction of glomeruli with mesangial widening in patients who received placebo.

- The measurements of standard renal histopathology in Study TKT003 correlate with renal function, and improvements in pathology correlate with improvements in renal function.

Cardiac Effects

- Replagal reduces cardiac mass in male patients with Fabry Disease. Study TKT005 demonstrated a significant reduction of cardiac mass compared with placebo, and Study TKT006 demonstrated a significant reduction of cardiac mass from baseline in an open label study. Replagal also reduced cardiac mass in female patients with Fabry Disease. Data from Study TKT014 demonstrated a significant reduction of cardiac mass from baseline in female patients.

- Replagal improved cardiac conduction system function in males in Studies TKT005 and TKT006. Replagal also improved cardiac conduction system function in female patients (Study TKT014).

Other Effects

- Replagal improves metabolic function as manifested by an improvement in patient weight.

- Replagal improves cerebrovascular blood flow.

- Replagal decreases Gb_3 levels in plasma and urine sediment. The potential use of these biomarkers as surrogates of clinical effectiveness is discussed in Section 5 of this briefing book.

4.3. The Effects of Replagal on Renal Disease

In this section, the following points are discussed:

- Two six-month placebo-controlled clinical studies were performed. In Study TKT003, Replagal stabilized renal function compared with placebo over six months. Study TKT010 did not demonstrate a difference in renal function between patients randomized to placebo and patients randomized to Replagal after six months of treatment.

- In Studies TKT006 and TKT011, long-term therapy with Replagal stabilized renal function after 2-2.5 years.

- Replagal improved renal pathology compared with placebo in Study TKT003. The measurements of renal pathology in Study TKT003 correlate with renal function, and improvements in pathology correlate with improvements in renal function.

- These changes in standard glomerular histopathology, as measured in Study TKT003, predict functional changes.

4.3.1. Renal Function: 6 Month Data

Renal function in Studies TKT003, TKT006, and TKT011 was measured by both creatinine clearance and GFR. Preliminary data for renal function as measured by GFR in Study TKT010 are presented below.

4.3.1.1. Creatinine Clearance; 6 Month Data

Study TKT003

The creatinine clearance results from Study TKT003 are shown in Table 9A.

Table 9A: Creatinine Clearance (mL/min) in TKT003

Visit	Replagal (patient number)	Placebo (patient number)
1st Baseline	103.1±7.6 (n = 14)	107.3±12.2 (n = 12)
2nd Baseline	109.3±9.1 (n = 12)	129.7±15.6 (n = 10)
Baseline Mean	105.3±7.5 (n = 14)	111.6±14.7 (n = 11)
Week 9	112.5±9.4 (n = 14)	105.6±15.0 (n = 11)
Week 17	111.7±9.9 (n = 12)	104.1±9.5 (n = 11)
Week 23	106.8±9.9 (n = 14)	100.7±16.1 (n = 10)
Week 24	103.1±7.5 (n = 14)	85.7±11.2 (n = 11)
Week 23/24 mean	105.0±8.3 (n = 14)	93.0±12.3 (n= 11)
Summary		
Baseline Mean	105.3±7.5 (n = 14)	111.6±14.7 (n = 11)
Week 23/24 Mean	105.0± 8.3 (n = 14)	93.0±14.7 (n = 11)
Change	–0.4±5.2 (n = 14)	–18.5±7.3 (n = 11)
p-value	0.051	

Mean ± SE

In Study TKT003, patients randomized to placebo had a significant decline in renal function, while six months of therapy with Replagal was associated with an initial stabilization of renal function (Table 9A). The p-value for the difference between patients treated with Replagal and patients treated with placebo was 0.051. This analysis includes all creatinine clearance samples that were collected during the Study.

Collections of 24-hour urine specimens for creatinine clearance estimation of GFR are often complicated by both over and under collections of urine samples. Over collection of a sample leads to over estimation of creatinine clearance, and under collection of a specimen results in under estimation of the creatinine clearance. Prior to unblinding Study TKT003, nephrologists at the NIH proposed an operational plan for excluding inaccurate collections. This plan called for the exclusion of collections in which the total urine creatinine (the total urine creatinine in a 24-hour specimen is constant for a given patient) was more than 35% different from the mean of the other five collections during the study for an individual patient.

Prior to unblinding the study, four collections were identified as either over or under collections. These included one patient at Week 9 in the Replagal treatment group and three patients at Week 23 and 24 in the placebo treatment group. FDA has pointed out that the differences between the Week 23 and Week 24 collections for two of these patients in the placebo group are physiologically implausible. We agree with this assertion. We believe that these differences are the result of under collections for these patients rather than any physiological changes.

In addition, prior to unblinding the study, one patient in the Replagal treatment group was prospectively excluded from the analysis; this patient suffered a renal infarct associated with the baseline renal biopsy and lost renal function following that biopsy as a result of the infarct.

Therefore, the data from Study TKT003 are appropriately analyzed as follows:

- Excluding the three under collections and one over collection of creatinine clearance samples (a total of 4 of 156 samples collected). Both over and under collections of creatinine clearance specimens are inaccurate estimates of creatinine clearance.

- Using Last Observation Carried Forward (LOCF) imputation for missing values. This allows direct comparison of the mean values at each time point in the study. Note: There were eleven missing specimens of 156 scheduled creatinine clearance collections in Study TKT003.

- Using the mean of both baseline creatinine clearance samples, and the mean of the Week 23 and 24 samples. Using the mean of two samples is more accurate.

- Excluding the patient who lost renal function as a result of the baseline renal biopsy related kidney infarction. This patient's decline in renal function was a result of the kidney biopsy and not the result of progression of disease.

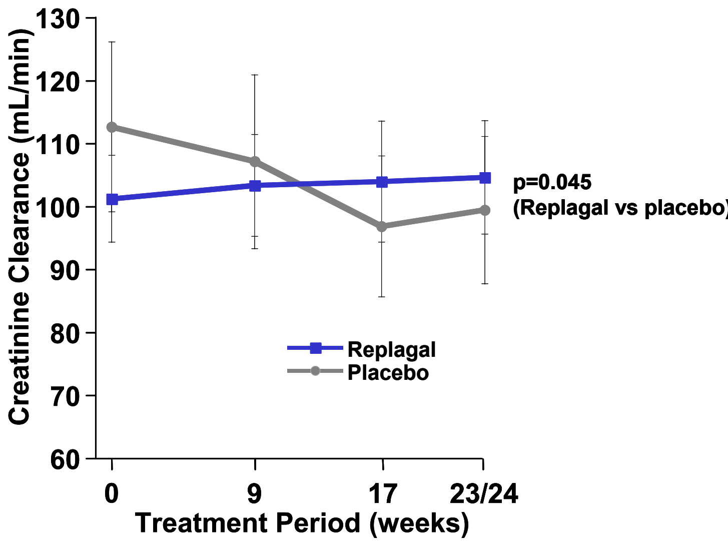
The result of this analysis is shown in Table 9B and Figure 8.

Table 9B: Creatinine Clearance (mL/min) in TKT003 – Alternate Analysis

	Replagal (n =13)	**Placebo (n = 12)**
Baseline mean	101.3±6.6	112.7±13.5
Week 9	103.4±8.1	107.2±13.8
Week 17	104.0±9.6	96.9±11.2
Week 23/24 Mean	104.7±8.7	99.5±11.7
p-value	0.045	

Mean ± SE

Figure 8: Creatinine Clearance in Study TKT003



As shown in Tables 9A and 9B, there is a progressive decline of renal function in patients randomized to placebo. This rate of decline is consistent with the natural history of disease (See Section 3.2.1). Patients randomized to Replagal, on the other hand, experienced a slight increase in renal function as measured by creatinine clearance during Study TKT003. The p-value for these differences was 0.045 and is similar to the results of the analysis including all of the data (p = 0.051).

4.3.1.2. Glomerular Filtration Rate; 6 Month Data

Study TKT003

The GFR results from Study TKT003 are shown in Table 10. The results for GFR in Study TKT003 were not statistically significant.

Table 10: GFR in Study TKT003

GFR (mL/min/1.73 m^2)	Replagal (n = 14)*	Placebo (n = 11)
Baseline	81.0±6.39	90.9±12.07
Week 24	72.2±4.31	71.1±9.92
Change to Week 24	–8.8±3.84	–19.8±7.11
p-value	0.245	

Mean ± SE

* If the one patient who lost renal function due to a renal hemorrhage following the baseline renal biopsy prior to receiving Replagal is excluded, baseline GFR is 77.2±5.57, the change to Week 24 is –6.2±3.1, and p=0.168

Study TKT010

The preliminary GFR results from Study TKT010 are shown in Table 11. The preliminary results for GFR in Study TKT010 were not statistically significant.

Table 11: GFR in Study TKT010

GFR (mL/min/1.73 m^2)	Replagal (n = 40)	Placebo (n = 40)
Baseline	89.6±4.2	82.9±4.7
Week 24	84.9±3.9	79.4±5.1
Change to Week 24	–4.7±2.1	–3.5±1.9
p-value	0.74	

Mean ± SE

4.3.1.3. Standard Renal Histopathology: Effects of Replagal

In addition to assessing clinical measures of efficacy, Study TKT003 also included measurements of the renal pathology of Fabry disease. Patients underwent renal biopsy at Baseline and at Week 24. Two pathologists from the Armed Forces Institute of Pathology reviewed the slides while blinded to treatment assignment, order of biopsy (i.e., whether the slide was taken from a Baseline or a Week 24 biopsy), and patient identification. A mean of 24.3 glomeruli were examined per biopsy. The pathologists review used four measures of standard histopathology:

- normal glomeruli,
- glomeruli with mesangial widening,
- glomeruli with segmental sclerosis, and
- obsolescent glomeruli.

Although this standard histopathology review was not included in the statistical analysis plan, the scoring system for standard histopathology was devised by the reviewing pathologists while they were blinded and before they scored the slides, and in that sense was prospectively established. (For a discussion of the procedures used for blinding and other aspects of the renal pathology assessments, please see Appendix 3.)

On two of the standard histopathology measures, the percentage of normal glomeruli and glomeruli with mesangial widening, Replagal was significantly more effective than placebo. There was an increase in the fraction of normal glomeruli and a decrease in the fraction of glomeruli with mesangial widening for patients treated with Replagal, but there was an increase in the fraction of glomeruli with mesangial widening and a decrease in the fraction of normal glomeruli for patients treated with placebo. These differences were statistically significant. Data on standard histopathology are summarized in Tables 12 and 13 below.

Table 12: Kidney Pathology - Normal Glomeruli

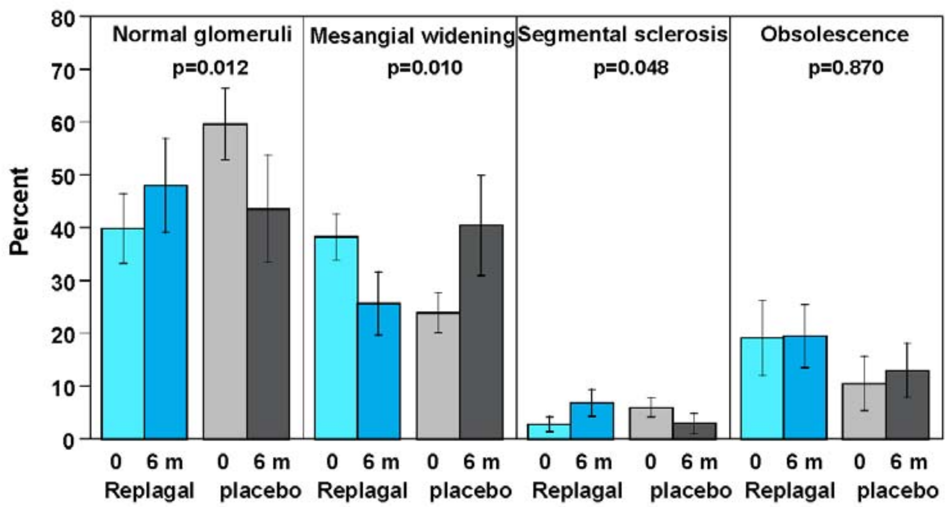
Kidney Pathology - Fraction of Normal Glomeruli	**Replagal (n=12)**	**Placebo (n=9)**
Baseline – Mean ± SE	0.399±0.066	0.596±0.068
Week 24 – Mean ± SE	0.480±0.089	0.436±0.101
Change from Baseline - Week 24 Mean±SE	0.082±0.044	−0.159±0.076
p-value	0.012	

Table 13: Kidney Pathology - Fraction of Glomeruli with Mesangial Widening

Kidney Pathology - Mesangial Widening	**Replagal(n=12)**	**Placebo (n=9)**
Baseline – Mean ± SE	0.382±0.043	0.239±0.038
Week 24 – Mean ± SE	0.257±0.060	0.404±0.095
Change from Baseline - Week 24 Mean ± SE	−0.125±0.050	0.165±0.077
p-value	0.010	

The summary data for the effect of Replagal on all four categories of renal pathology are presented in Figure 9.

Figure 9: TKT003 Kidney Pathology Results



There was a small difference in the number of glomeruli with segmental sclerosis. There was a slight decrease in the fraction of glomeruli with segmental sclerosis in the placebo patients and a slight increase in patients treated with Replagal (p=0.048), although the numbers of glomeruli were small in these two categories. However, there was an increase in obsolescent glomeruli in patients randomized to placebo, suggesting that the decrease in the number of glomeruli with segmental sclerosis represented glomeruli that had worsened and progressed to obsolescent glomeruli during the study. Based on the severity of segmental sclerosis and glomerular obsolescent pathology, it is unlikely that these two aspects of the disease would be able to be reversed with therapy.

4.3.1.4. Standard Renal Histopathology as a Surrogate Marker in Fabry Disease

The importance of the pathology data from Study TKT003 is strengthened by the correlation of histopathology with renal function in Fabry Disease. Figures 10, 11, and 12 demonstrate the correlation of renal pathology as measured in Study TKT003 with renal function. In these graphs a total of 25-paired measurements of renal function and renal pathology at baseline in Study TKT003 are placed in a scatter plot.

Figure 10A: GFR vs the Fraction of Normal Glomeruli (%): Regression Line and 95% C.I.

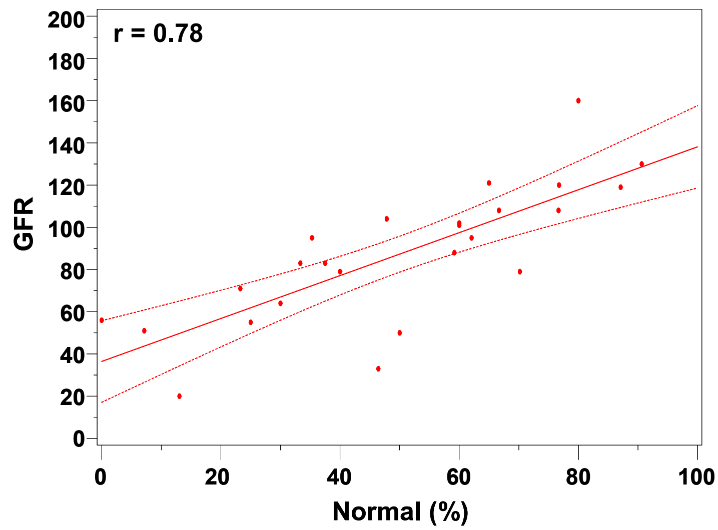


Figure 10B: Creatinine Clearance vs the Fraction of Normal Glomeruli (%): Regression Line and 95% C.I.

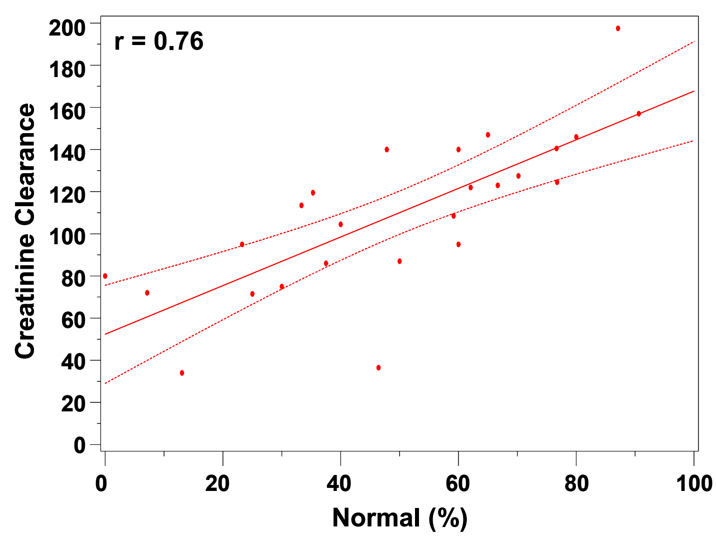


Figure 11A: GFR vs the Fraction of Glomeruli with Segmental Sclerosis and Obsolescence (%): Regression Line and 95% C.I

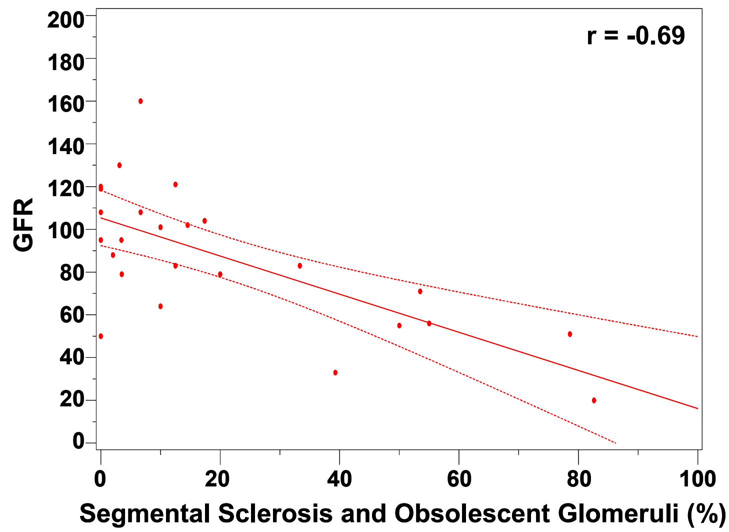


Figure 11B: Creatinine Clearance vs the Fraction of Glomeruli with Segmental Sclerosis and Obsolescence Glomeruli (%): Regression Line and 95% C.I.

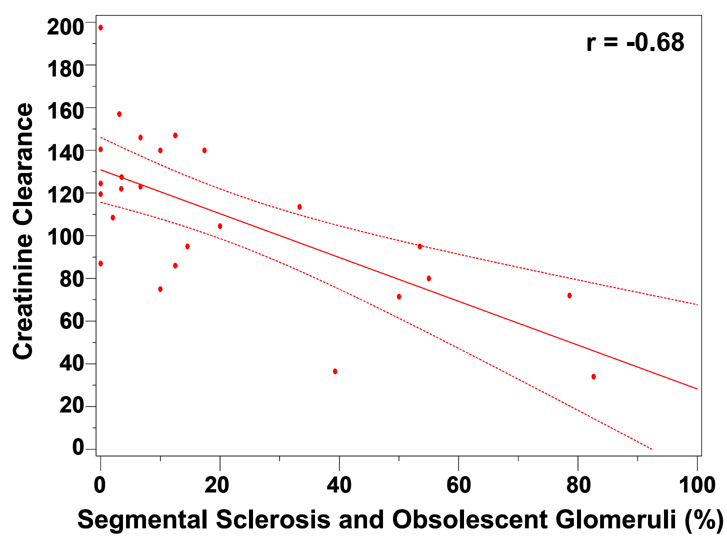


Figure 12A: GFR vs the Fraction of Glomeruli with Mesangial Widening (%): Scatter Plot

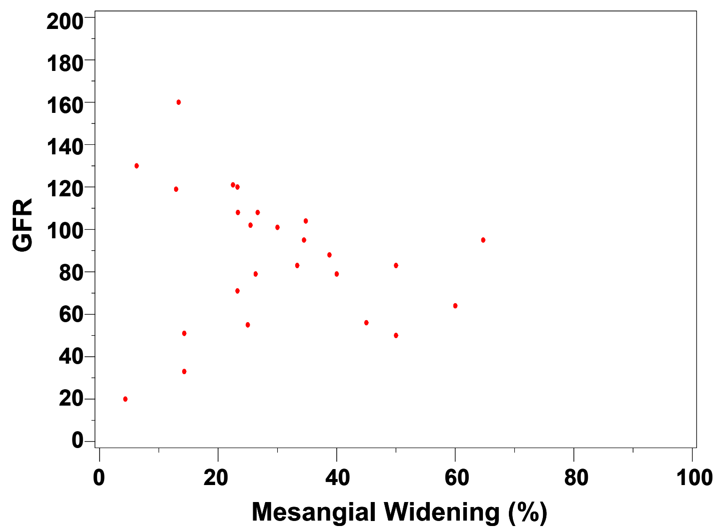
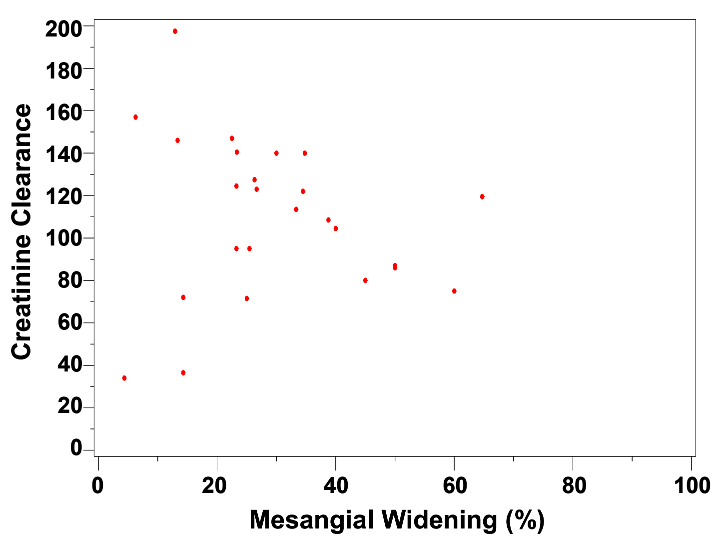


Figure 12B: Creatinine Clearance vs the Fraction of Glomeruli with Mesangial Widening (%): Scatter Plot



As shown in Figures 10A and 10B above, there is a positive linear correlation of both GFR (r = 0.78) and creatinine clearance (r = 0.76) with the fraction of normal glomeruli in patients' renal biopsies. That is, the higher the fraction of normal glomeruli, the higher the level of renal function. As shown in Figures 11A and 11B, there is an inverse linear correlation of GFR (r = -0.69) and creatinine clearance (r = -0.68) with the fraction of glomeruli with segmental sclerosis and obsolescence. As these measures of severe pathology increase, there is a progressive decrease in renal function. As pathology worsens, so does renal function.

The change in the fraction of glomeruli with mesangial widening also correlates (albeit in a complex fashion) with renal function and is consistent with the progression of renal pathology from normal glomeruli to glomeruli with mesangial widening to glomeruli with segmental sclerosis and obsolescence. At normal GFR, there is a relatively small fraction of glomeruli with mesangial widening. As GFR decreases, there is a corresponding increase in the fraction of glomeruli with mesangial widening. Then, as GFR or creatinine clearance falls into the range of ESRD, as shown in Figures 12A and 12B, glomeruli with mesangial widening likely progress to sclerosis and obsolescence. In other words, a small fraction of glomeruli with mesangial widening is consistent with normal or nearly normal GFR as well as severely compromised GFR. A large fraction of glomeruli with mesangial widening correlates with impaired renal function and chronic renal insufficiency. Hence, a glomerulus with mesangial widening could either worsen to become sclerosed or obsolescent as GFR decreases, or, following therapy with Replagal, revert to normal, with a concomitant improvement in GFR.

These data demonstrate that the assessment of standard glomerular histopathology as performed in Study TKT003 correlates with renal function. Improvements in glomerular histopathology associated with therapy with Replagal correlated with and heralded improvements in GFR in these patients. As such, standard glomerular histopathology correlates with renal function and is reasonably likely to predict clinical benefit in Fabry Disease.

4.3.2. Renal Function: Long-Term Data

4.3.2.1. Creatinine Clearance

The results for creatinine clearance of the combined three studies are presented in Table 14. In this Table, the patients initially randomized to placebo in Study TKT003 received Replagal for two years in Study TKT006 and TKT011; the baseline value for these patients is defined as the beginning of Study TKT006 (end of Study TKT003), when they first received Replagal. For patients initially randomized to Replagal in Study TKT003, the 2.5-year period on therapy is compared to the initial baseline in Study TKT003 (Month zero).

Table 14. Creatinine Clearance: Studies TKT003, TKT006, and TKT011

Month of Treatment / Study	**Mean±SE*** (mL/min)	**p-value**
Patients Treated with Placebo in TKT003 and Replagal in TKT006 and TKT011 (n=11)		
Month 0 / Baseline of TKT003 (n=11)	111.6±14.73	
Month 6 / End of TKT003 (n=11)	93.1±12.29	
Month 12 / TKT006 (n=11)	94.7±16.14	-
Month 18 / TKT006 (n=10)	104.0±16.04	
Month 24 / TKT011 (n=10)	120.8±16.01	
Month 30 / TKT011 (n=10)	107.2±17.69	
Change - Baseline to End of TKT003 (6 months placebo treatment, n=11)	−18.6±7.25	0.029
Change - End of TKT003 (Month 6) to:		
Month 12 (6 months Replagal treatment, n=11)	1.7±8.82	0.853
Month 18 (1 year Replagal treatment, n=10)	11.2±7.93	0.193
Month 24 (1.5 years Replagal treatment, n=10)	29.6±9.60	0.013
Month 30 (2 years Replagal treatment, n=10)	16.0±12.80	0.244
Patients Treated with Replagal in TKT003, TKT006, and TKT011 (n=13)		
Month 0 / Baseline of TKT003 (n=13)	104.2±8.04	
Month 6 / End of TKT003 (n=13)	103.0±8.75	
Month 12 / TKT006 (n=13)	96.3±9.43	-
Month 18 / TKT006 (n=13)	99.2±10.19	
Month 24 / TKT011 (n=12)	106.2±11.49	
Month 30 / TKT011 (n=12)	112.2±13.61	
Change - Baseline of TKT003 (Month 0) to:		
Month 6 (6 months Replagal treatment, n=13)	−1.2±5.52	0.833
Month 12 (1 year Replagal treatment, n=13)	−7.9±8.88	0.390
Month 18 (1.5 years Replagal treatment, n=13)	−5.0±6.28	0.441
Month 24 (2 years Replagal treatment, n=12)	−0.4±6.78	0.952
Month 30 (2.5 years Replagal treatment, n=12)	5.5±9.79	0.586

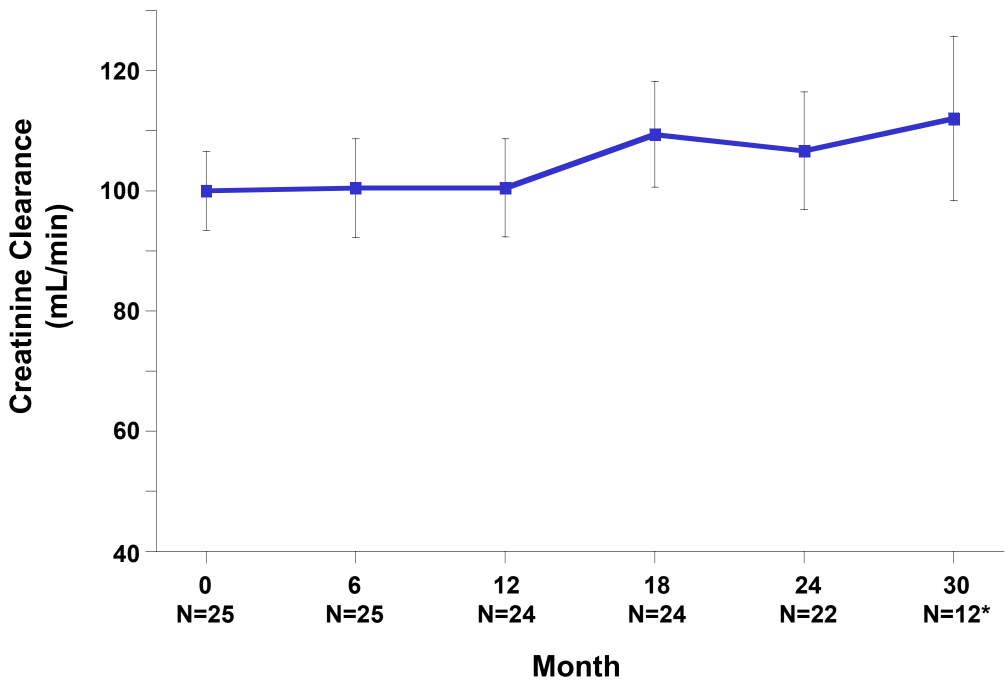
* Due to the differences in patient number at the different time points, the mean changes cannot be derived by subtracting the mean GFR values listed above

As described above, the patients initially randomized to placebo in Study TKT003 had a significant decline in renal function over six months in that study.

However, when these same patients were crossed over to therapy with Replagal, the decline in renal function seen in Study TKT003 was halted. Over the first 6 months of therapy on Study TKT006, there was stabilization in renal function with a mean increase of 1.7 mL/min (p=0.853). At the end of a full year of therapy, improvement in renal function was seen, with a net increase of 11.2 mL/min in creatinine clearance (p=0.193). After further therapy with Replagal in Study TKT011, the mean increase in creatinine clearance was 29.6 mL/min (p=0.013) at month 24 and was 16.0 mL/min (p=0.244) at month 30. Thus, while receiving placebo, these patients experienced a decline in renal function as measured by creatinine clearance, but over two years of therapy with Replagal, there was an improvement in these patients' renal function as measured by creatinine clearance which was statistically significant at month 24 (18 months of therapy) and remained improved at month 30 (2 years of therapy).

Patients who were initially treated with Replagal in Study TKT003 also demonstrated initial stabilization followed by a slight improvement in renal function during approximately 2.5 years of treatment. In Study TKT011, the additional year of treatment after Study TKT006 (2.5 years total) with Replagal, the net mean increase in creatinine clearance was 5.5 mL/min (p=0.586). Thus, the initial stabilization followed by improvement of renal function seen in patients treated with Replagal in both Studies TKT003 and TKT006 has been confirmed and extended by the analysis of the effect of Replagal therapy over the total 30-month period. More importantly, treatment with Replagal has halted the decline in renal function associated with the natural history of Fabry Disease (see Section 3.2.1).

Figure 13: Combined Creatinine Clearance in Studies TKT003, TKT006, and TKT011: 2 to 2.5 Years of Therapy with Replagal



In Figure 13, time zero is defined as the time at which patients initiated therapy with Replagal. As noted above, the patients originally randomized to Replagal in Study TKT003 have received Replagal for 2.5 years, and the patients originally randomized to placebo in study TKT003 have received Replagal for two years. As demonstrated in Figure 13, therapy with Replagal is initially associated with a stabilization of renal function over six to twelve months. This is followed by a progressive improvement (not statistically significant) in renal function over the subsequent twelve to eighteen months. These data demonstrate that 2 to 2.5 years of therapy with Replagal is associated with long-term stabilization of renal function in patients with Fabry Disease.

4.3.2.2. Glomerular Filtration Rate (GFR)

The statistical analyses for GFR in Studies TKT003, TKT006 and TKT011 are shown in Table 15.

Table 15: Change from Baseline in Glomerular Filtration Rate

Month of Treatment / Study	Mean±SE* ($mL/min/1.73m^2$)	p-value
Patients Treated with Placebo in TKT003 and Replagal in TKT006 and TKT011 (n=11)		
Month 0 / Baseline of TKT003 (n=10)*	98.0±10.80	-
Month 6 / End of TKT003 (n=10)	78.2± 7.65	
Month 18 / TKT006 (n=10)	95.4±10.76	
Month 30 / TKT011 (n=7)	86.7±15.03	
Change - Baseline to End of TKT003 (6 months placebo treatment, n=10)	–19.8±7.87	0.033
Change - End of TKT003 (Month 6) to:		
Month 18 (1 year Replagal treatment, n=10)	17.2± 6.41	0.025
Month 30 (2 years Replagal treatment, n=7)	12.7±12.92	0.363
Patients Treated with Replagal in TKT003, TKT006, and TKT011 (n=13)		
Month 0 / Baseline of TKT003 (n=13)	79.9± 6.80	-
Month 6 / End of TKT003 (n=13)	70.8±4.39	
Month 18 / TKT006 (n=13)	72.3±7.07	
Month 30 / TKT011 (n=9)	84.2±11.21	
Change – Baseline of TKT003 (Month 0) to:		
Month 6 (6 months Replagal treatment, n=13)	–9.2±4.13	0.047
Month 18 (1.5 years Replagal treatment, n=13)	–7.6±4.80	0.138
Month 30 (2.5 years Replagal treatment, n=9)	–4.1±7.28	0.588

* Due to the differences in patient number at the different time points, the mean changes cannot be derived by subtracting the mean GFR values listed above.

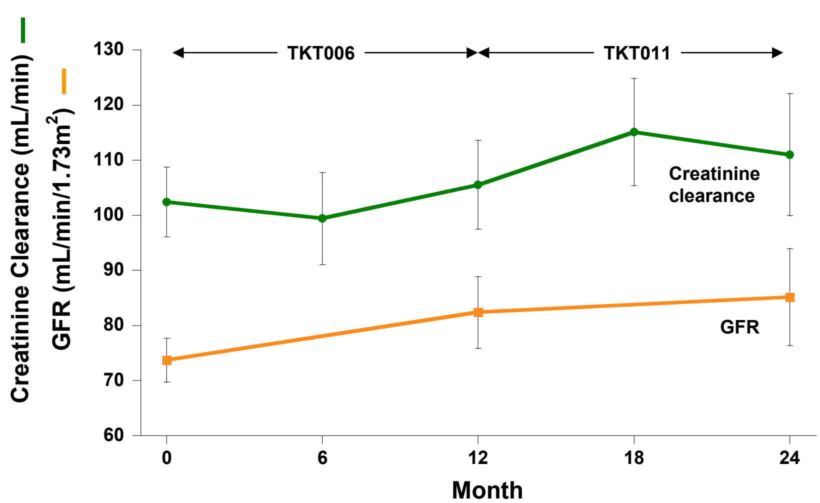
As shown in Table 15, during the six months of Study TKT003, patients randomized to placebo had a significant decline in GFR as compared to baseline (p=0.033). However, when these patients were crossed over to therapy with Replagal, improvement in renal function was observed. Over the one year of therapy in Study TKT006, there was a mean increase in GFR of 17.2 $mL/min/1.73m^2$ (p=0.025). An additional year of therapy (2 years total) with Replagal in Study TKT011 resulted in a net increase of 12.7 $mL/min/1.73m^2$ in GFR (p=0.363). Thus, the significant decline in renal function associated with 6 months of therapy with placebo was halted and reversed by 1 year of therapy with Replagal; the improvement was maintained during an additional year of Replagal therapy.

As shown in Table 15, during the six months of Study TKT003, patients randomized to Replagal had a significant decline in GFR as compared to baseline (p=0.047), though this decline is half that observed in the placebo group. A single patient who lost renal function due to a renal infarct following the baseline renal biopsy prior to receiving Replagal exaggerates the decline in the Replagal group. At the end of an additional year of therapy in Study TKT006, GFR improved slightly, and after one more year of therapy in Study TKT011 (2.5 years total) GFR improved further. Over the entire 2.5 years of therapy in the nine patients who completed Month 30, there was a slight decline in GFR of 4.1mL/min/1.73m^2. Overall the change after 2.5 years of therapy was not significantly different from baseline (p=0.588).

4.3.2.3. Renal Function: Long-Term Data - Conclusions

The effect of Replagal on renal function as measured by both creatinine clearance and GFR in studies TKT006 and TKT011 (two years of therapy in all patients studied) is presented in Figure 14 below.

Figure 14: Effect of Replagal on Renal Function: Two Years of Therapy in Studies



These data demonstrate a progressive improvement in renal function over two years. These data also demonstrate that the results for creatinine clearance and GFR are consistent. (Creatinine clearance values are usually higher than GFR values due to the renal tubular secretion of creatinine.) Over the two years of therapy in studies TKT006 and TKT011 there is a progressive improvement in renal function as measured by both creatinine clearance and GFR. The changes were statistically significant for GFR after one year ($p=0.037$) and were maintained after two years ($p=0.168$). For creatinine clearance, the results were statistically significant at 18 months ($p=0.051$) and were maintained at 2 years ($p=0.229$).

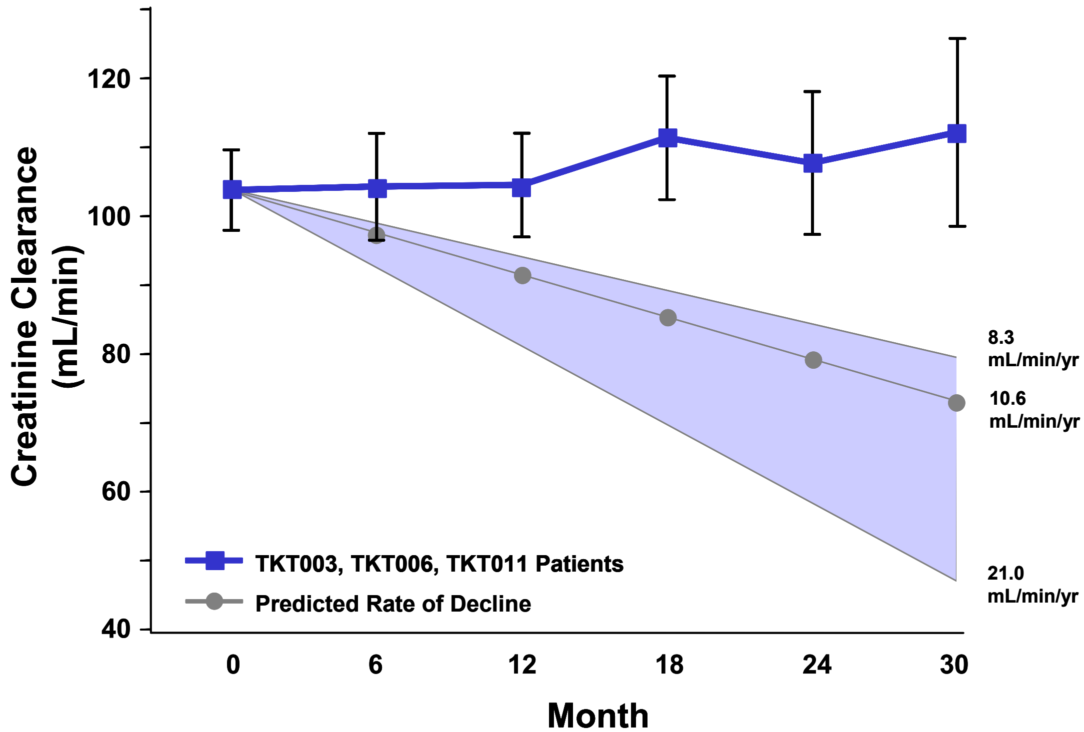
4.3.3. Serial Measurements of Renal Function in TKT Clinical Studies: Comparison to the Natural History Data

Combining data from studies TKT003, TKT005 and TKT010 the mean rate of decline in placebo patients is approximately 8.3 mL/min/yr (see Table 2). As discussed in Section 3.2.1 above, including all of the individual patients reported in the literature, the 14 patients reported in the series by Branton *et al*., and the TKT placebo patients, the mean rate decline of renal function in patients with Fabry Disease in their mid-30s is on average 10.6 mL/min/yr.

The mean GFR at baseline in Study TKT003 was 86.7±31.8 mL/min/1.73m^2. The mean creatinine clearance at Baseline was 108.1±7.6 mL/min. After 2 to 2.5 years of therapy in Studies TKT003, TKT006, and TKT011, as measured by either GFR or creatinine clearance, renal function has improved in these patients. If Replagal had no effect on renal function, then, based on the natural history data, renal function in these patients should have declined by approximately 26.5 mL/min over 2.5 years (10.6 mL/min/yr multiplied by 2.5 years). Patients treated with Replagal have not deteriorated during this time. The results of 2 to 2.5 years of therapy demonstrate that Replagal halts the progression of renal deterioration of Fabry Disease.

These results are presented graphically in Figure 15.

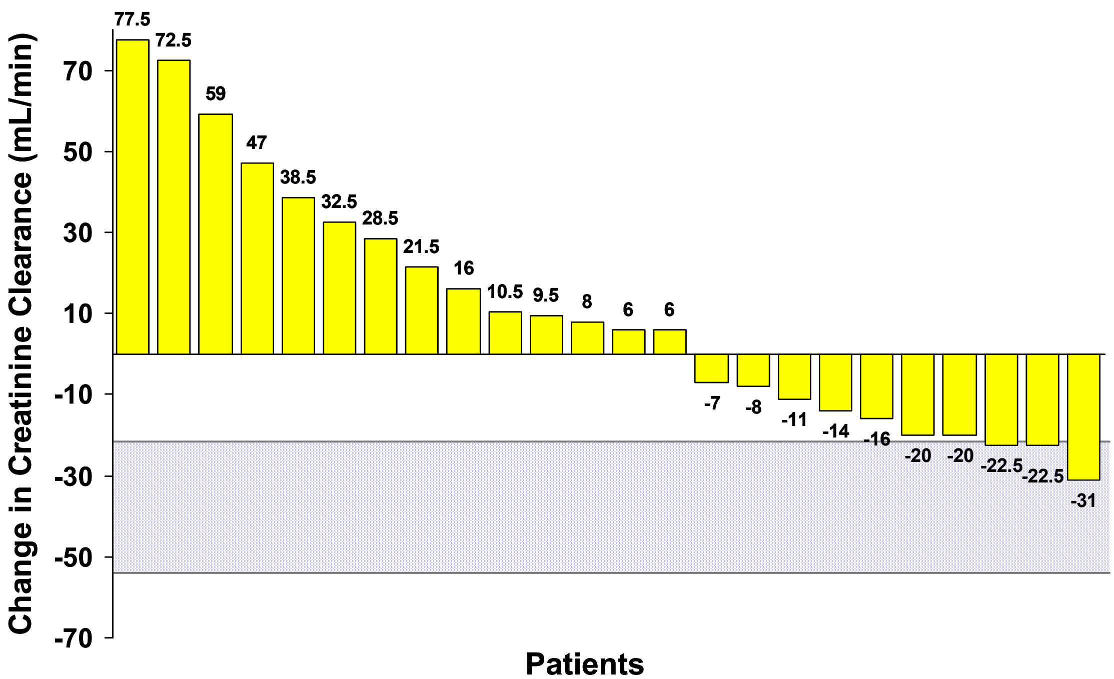
Figure 15: Combined Creatinine Clearance in Studies TKT003, TKT006 and TKT011 (2 to 2.5 years of therapy with Replagal) Compared to Predicted Rates of Decline



In Figure 15, the improvement in creatinine clearance in Studies TKT003, TKT006, and TKT011 is reproduced from Figure 13 above. In addition, Figure 15 shows the predicted rate of decline of renal function over the similar time period based on the mean rate of decline of 10.6 mL/min/yr from reports in the literature. The predicted rate of decline includes the range of 8.3-21 mL/min/yr based on individual case reports in the literature, the series by Branton *et al*. **[18]**, and the TKT003, TKT005, and TKT010 placebo patients.

More than half (14 of 24) of the patients who have received Replagal for 24 to 30 months have improved renal function as measured by creatinine clearance. In addition, compared with the expected rate of decline of at minimum of 8.3 mL/min/yr, at least 19 of 24 patients have either improved renal function or renal function that has declined at lower rate than expected based on the natural history of disease. Therefore, the majority of patients who have received Replagal have responded to therapy based on either improved renal function or a decrease in the rate of decline of renal function based on the expected rate of decline. Individual patient responses are shown in Figure 16.

Figure 16: Individual Creatinine Clearance Patient Data After Long-Term Therapy with Replagal



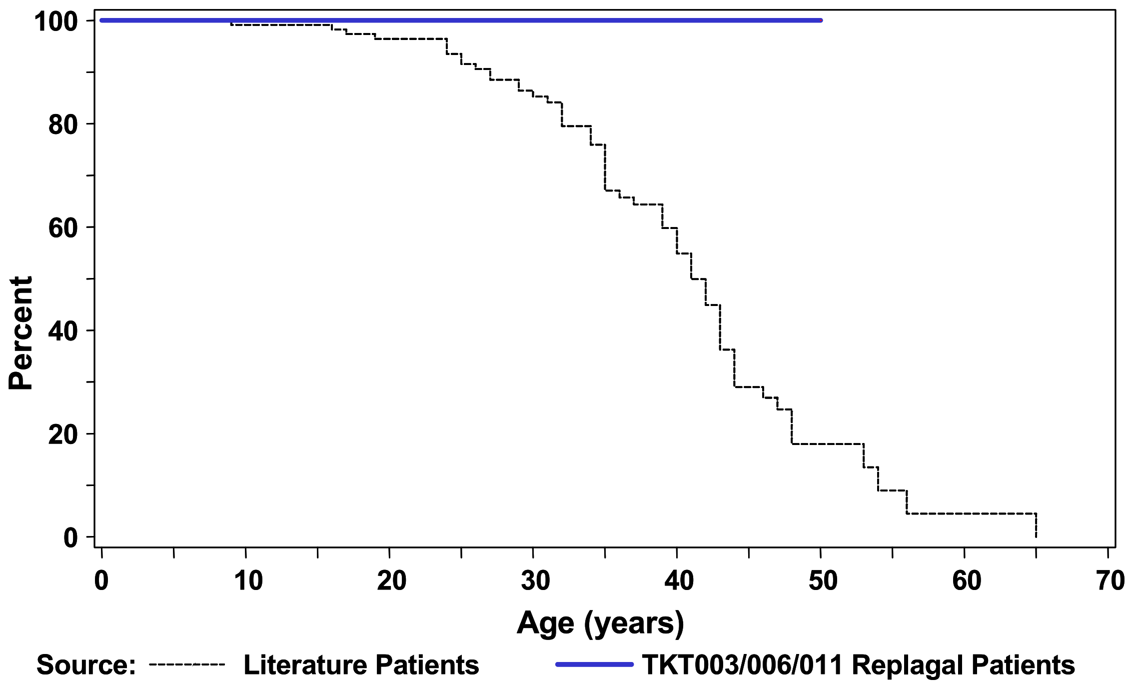
4.3.4. ESRD During Long-Term Clinical Studies with Replagal: Comparison To Natural History

The incidence of ESRD and the comparison to historical control patients was assessed in Study TKT011.

None of the patients who received Replagal during Studies TKT003, TKT006, and TKT011 has progressed to ESRD. These patients began Study TKT003 at an average age of approximately 34.2 years. After 2.5 years in the three clinical studies, none of the patients treated with Replagal has progressed to ESRD, and their mean age is approximately 38 years. Figure 17 below presents a Kaplan-Meier analysis of the time to progression to ESRD in the patients enrolled in Studies TKT003, TKT006, and TKT011 (n=25) compared with untreated historical control patient data derived from the natural history literature study described in Section 3.2.1 of this document.

Only one patient in the combined studies who was randomized to placebo in study TKT003 progressed to ESRD.

Figure 17: ESRD: Patients in Studies TKT003, TKT006, and TKT011 Versus Historical Control Patients



These data suggest that Replagal has delayed the time to progression to ESRD and may prevent the progression to ESRD in these patients compared with historical controls from the literature.

To determine if patients treated with Replagal are significantly different from historical control patients from the literature, we performed a risk assessment analysis to determine the probability that no patients treated with Replagal have progressed to ESRD.

For each patient in Studies TKT003/006/011, the conditional probability of progressing to ESRD was calculated given the patient's age and the condition that patients were not in ESRD at the beginning of Study TKT003 (one of the selection criteria). The control curve for this analysis consisted of the patients described in the literature above (see Figure 17). For example, a patient who is 38 years old would have a probability of not being in ESRD of approximately 0.66. Using the control curve from Figure 17, the probability of progressing to ESRD can be calculated for this patient over a period of observation. For example, if this patient were followed for four years, when he was 42 years old, the patient's probability of not being in ESRD would be approximately 0.52. Thus, the probability of this patient progressing to ESRD over four years, given the condition of not being in ESRD age 38, would be [(0.66 – 0.52)/0.66] or 0.21.

In this fashion, the probability of progressing to ESRD for each patient randomized to placebo in Study TKT003 can be calculated. Then, the number of expected events (where an event is defined as progression to ESRD) expected in the placebo population in Study TKT003 is calculated from the sum of the individual probabilities.

In addition, the number of expected events in all patients treated with Replagal can also be calculated. The period of observation is defined as the period of therapy with Replagal. That is, placebo patients in Study TKT003 began Replagal in Study TKT006 and patients randomized to Replagal in Study TKT003 began Replagal with that study. The period of observation continues to the present time for 25 of the 26 patients initially enrolled in Study TKT003. These data are shown in Table 16.

Table: 16. Progression to ESRD: Studies TKT003/006/011

Population	Observation	Expected Events	Observed Events
TKT003 – placebo (n = 12)	6 months	0.7	1
TKT003/006/011 – Replagal (n = 24)*	3.5 to 4 years	4.7	0

* Excludes the one patient who progressed to ESRD while receiving placebo in Study TKT003.

This analysis suggests that among 12 patients initially randomized to placebo in Study TKT003, the sum of the individual probabilities of progression to ESRD = 0.7. That is, in the 6-month period of Study TKT003, 0.7 patients would have been expected to progress to ESRD during the 6 months of that study. The number of observed events (patients progressing to ESRD) was 1.

In contrast, over the 3.5 to 4 years of therapy with Replagal in Studies TKT003/006/011, these data suggest that there would be 4.7 expected events. That is, 4.7 patients would have been expected to progress to ESRD during Studies TKT003/006/011 over the 3.5 to 4 years of these studies. The number of patients who progressed to ESRD is 0.

Finally, the probability of a patient not progressing to ESRD can be calculated as [1 – probability (ESRD)]. These probabilities can then be multiplied, since they are independent events, to determine the probability of no one progressing to ESRD during therapy with Replagal. The probability of observing zero events in a patient population with a current mean age of approximately 38 years over 3.5 to 4 years of therapy with Replagal is 0.006.

This analysis, although based on historical control data from the literature, suggests that Replagal may delay and potentially prevent progression to ESRD in patients with Fabry Disease.

4.3.5. The Effects of Replagal on Renal Disease: Conclusions

The effect of Replagal on kidney disease in subjects with Fabry Disease has been assessed in four clinical studies (TKT003, TKT006, TKT011, and TKT010).

Study TKT003 initially demonstrated stabilization of renal function associated with Replagal therapy compared with a decline in renal function in patients randomized to placebo. The initial decline in renal function associated with placebo in Study TKT003 is consistent with the natural history of disease. Long-term data have demonstrated that treatment with Replagal resulted in improvements in renal function after up to 2.5 years of therapy. Study TKT010, however, did not demonstrate a difference between Replagal and placebo on renal function over six months.

No subject who has received Replagal in these studies has progressed to end stage renal disease. In comparison with the natural history data, there is a substantial difference in terms of progression to ESRD between the Replagal treated patient population in these studies and the untreated patient population in the natural history database. These data suggest that Replagal may delay and possibly prevent the progression to ESRD in patients with Fabry Disease.

Finally, Study TKT003 demonstrated an improvement in kidney pathology in patients treated with Replagal compared to a worsening of kidney pathology in patients treated with placebo. Patients randomized to Replagal had a significant decrease in the fraction of glomeruli with mesangial widening and a significant increase in the fraction of glomeruli that were normal. These data suggest that the pathologic finding of mesangial widening in patients with Fabry Disease can be reversed by therapy with Replagal. In essence, some glomeruli with mesangial widening in the Replagal-treated patients became normal. These changes correlated with improvements in renal function and are therefore reasonably likely to predict clinical benefit.

Taken together, the data from studies TKT003, TKT006, and TKT011 demonstrate that Replagal improves renal pathology, initially stabilizes and subsequently improves renal function, and delays or may prevent the progression to ESRD for patients with Fabry Disease.

4.4. The Effects of Replagal on Cardiac Disease

In three different patient populations (the NIH patients in Studies TKT003 and TKT006, the RFH patients in Study TKT005, and the University of Mainz patients in Study TKT014), therapy with Replagal has been demonstrated to decrease cardiac mass and improve cardiac conduction system function.

4.4.1. Study TKT005

As described in Section 4.1.2 (above), Study TKT005 was a randomized, double blind, placebo controlled trial conducted over 6 months. Patients were included in this study based on echocardiographically determined evidence of left ventricular hypertrophy. The primary efficacy endpoint was the measurement of Gb_3 content in endomyocardial biopsy specimens. Additional efficacy endpoints included an assessment of the effect of enzyme replacement therapy on cardiac mass as determined by MRI and echocardiography. An overview of the cardiac outcome data from study TKT005 is shown in Table 17.

Table 17: Study TKT005 Cardiac Outcomes

Parameter*	Replagal	Placebo
Cardiac Gb_3 Content (nmol/μg protein)		
Baseline	0.71±0.18 (n=6)	0.58±0.08 (n=8)
Change from Baseline – Week 24 % Change	–0.13±0.16 –19%	0.05±0.08 9%
p-value	0.42	
Left Ventricular Mass by MRI (grams)		
Baseline	276±19 (n=7)	248±26 (n=7)
Change from Baseline – Week 24 % Change	–11.5±11.2 –4.2%	21.8±5.9 +8.8%
p-value	0.041	
Left Ventricular Mass by Echo (grams)		
Baseline	327±22 (n=7)	342±40 (n=8)
Change from Baseline – Week 24 % Change	–20±27 –6.2%	22±20 +6.3%
p-value	0.26	

*All values are Mean±SE

Although patients treated with Replagal had a 19% decline in cardiac Gb_3 content and patients treated with placebo had a 9% increase in cardiac Gb_3 content, these differences were not statistically significant (p = 0.42).

As shown in Table 17 above, the patients with severe cardiomyopathy at baseline in Study TKT005 had markedly elevated baseline cardiac masses, with means of 248-276 grams (normal cardiac mass is approximately 150 grams). Compared with placebo, therapy with Replagal resulted in a statistically significant decrease in cardiac mass. Patients treated with Replagal had an approximately 4% decrease in their cardiac mass as measured by MRI, while patients randomized to placebo had an approximately 9% increase in cardiac mass as measured by MRI (p=0.041). The results from the echocardiographic analysis of LV mass, although not statistically significant, were consistent with the result obtained from MRI.

4.4.2. Studies TKT003 and TKT006

Measurements of cardiac mass were also performed in Studies TKT003 and TKT006. The change from Baseline to Week 52 in cardiac mass as measured by MRI was one of the primary endpoints of Study TKT006. An overview of the LV Mass (determined by MRI) data from studies TKT003 and TKT006 is shown in Table 18.

Table 18: Left Ventricular Mass Data from Studies TKT003 and TKT006

Assessment	Mean±SE(g)	p-value
Patients Treated with Placebo in TKT003 and Replagal in TKT006 (n=11)		
Baseline of TKT003 (n=11)	211.8±12.58	
End of TKT003 (Week 24, n=11)	215.9±11.20	
Week 27 of TKT006 (n=11)	194.5±13.10	-
Week 52 of TKT006 (n=10)	181.25±9.68	
Change - Baseline to Week 24 of TKT003 (24 weeks of treatment with placebo, n=11)	4.1±5.66	0.486
Change - End of TKT003 to Week 27 of TKT006 (6 months of treatment with Replagal, n=11)	–21.5±10.02	0.058
Change - End of TKT003 to Week 52 of TKT006 (1 year of treatment with Replagal, n=10)	–27.65±10.06	0.023
Patients Treated with Replagal in TKT003 and TKT006 (n=14)		
Baseline of TKT003 (n=14)	226.1±17.41	
End of TKT003 (Week 24, n=14)	229.6±16.97	-
Week 27 of TKT006 (n=14)	207.0±13.70	
Week 52 of TKT006 (n=14)	207.9±18.33	
Change - Baseline to Week 24 of TKT003 (first 24 weeks of treatment with Replagal, n=14)	3.5±2.66	0.211
Change - End of TKT003 to Week 27 of TKT006 (additional 6 months of treatment with Replagal, n=14)	–22.7±4.20	<0.001
Change - End of TKT003 to Week 52 of TKT006 (additional 1 year of treatment with Replagal, n=14)	–21.7±4.70	<0.001
Change - Baseline of TKT003 to Week 52 of TKT006 (18 months of treatment with Replagal, n=14)	–18.2±5.56	0.006

Although there were no inclusion criteria for patients with elevated cardiac mass in Study TKT003, the patients did have elevated cardiac masses at Baseline (means of 211.8 and 226.1 grams for placebo and Replagal groups respectively; normal approximately 150 grams).

There was a significant effect of Replagal therapy on cardiac mass as determined by MRI. Patients treated with Replagal for 18 months as well as those patients crossed over from treatment with placebo experienced statistically significant reductions from baseline in left ventricular mass measured by MRI. Patients initially randomized to placebo in Study TKT003 experienced an increase in cardiac mass. However, one year of therapy with Replagal resulted in an approximately 13% reduction in cardiac mass ($p=0.023$; change from Baseline). Similarly, patients originally randomized to receive Replagal in Study TKT003 also had statistically significant reductions in cardiac mass over the 18 months of therapy with Replagal. Over 12 to 18 months, these patients had an approximate 10% decline in cardiac mass ($p=0.006$).

Finally, in Study TKT003 there was a significant effect of Replagal therapy on cardiac conduction system function. Patients randomized to Replagal had a decrease in QRS complex duration, while patients randomized to placebo had an increase in QRS complex duration. The difference was statistically significant ($p=0.047$). In addition, one patient randomized to Replagal in Study TKT003 had a right bundle branch block pattern revert to normal during the study. The decreases in QRS duration observed in Study TKT003 were maintained in Study TKT006, and the patient whose right bundle branch block reverted to normal in Study TKT003 maintained this improvement during Study TKT006.

4.4.3. Study TKT014

As described in Section 4.1.3 (above), Study TKT014 was an open label trial conducted in female patients with Fabry Disease. Efficacy endpoints included an assessment of the effect of enzyme replacement therapy on cardiac mass as determined by echocardiography. An overview of the cardiac outcomes in Study TKT014 is presented in Table 19. All echocardiographic examinations in Study TKT014 were performed by one experienced cardiologist, digitally saved, and later analyzed blinded to the name and clinical status of the patients. A summary of changes in left ventricular mass, left ventricular mass index, interventricular septal thickness and left ventricular posterior wall thickness are presented in Table 19.

Table 19: Study TKT014 Cardiac Outcomes

Parameter	Change from Baseline to Week:		
	13 (n=15)	**27 (n=11)**	**41 (n=7)**
LVM (g)	−10.7±9.59 (0.285)	−38.5±9.73 (0.003)	−42.7±16.27 (0.039)
LV Mass Index (g)	−5.5±5.94 (0.372)	−23.0±5.78 (0.003)	−25.2±8.12 (0.021)
Intraventricular Septal Thickness (mm)	−1.0±0.23 (<0.001)	−2.2±0.38 (<0.001)	−2.2±0.60 (0.011)
LV Posterior Wall Thickness (mm)	−0.6±0.55 (0.311)	−1.5±0.58 (0.028)	−2.2±1.10 (0.093)
ECG-QRS Complex Duration (msec)	−5.5±2.96 (0.086)	−8.7±2.60 (0.007)	−3.6±1.83* (0.121)

* n=5

The data presented in Table 19 shows a statistically significant decline in cardiac mass from baseline to Week 27. After six months of therapy, there were statistically significant declines in left ventricular mass (p=0.003), left ventricular mass index (p=0.003), interventricular septal thickness (p<0.001), and left ventricular posterior wall thickness (p=0.028). These changes represented an approximate 15% decline in cardiac mass in these patients over six months.

Each of the patients who began the study with an elevated cardiac mass index (>125 g/m^2) experienced a decline in cardiac mass over the course of this study. Additionally, the four patients who received Replagal for the longest time (55 weeks) all experienced a decline in cardiac mass over one year of therapy with Replagal. These studies are consistent with previous studies of the effects of Replagal on cardiac mass in male patients with Fabry Disease. Both Studies TKT005 and TKT006 demonstrated that therapy was associated an approximately 10% decline in cardiac mass.

Therefore, the effects of Replagal on the heart in female patients are similar to the effects of Replagal on the heart in male patients with Fabry Disease.

The mean QRS interval decreased progressively from baseline through Week 41. A statistically significant mean decrease from baseline (p=0.007) was observed at Week 27.

In summary, these results demonstrate that therapy with Replagal improves cardiac disease in female patients with Fabry Disease.

4.4.4. The Effects of Replagal on Cardiac Disease: Conclusions

These four studies in three independent patient populations have all demonstrated similar results: six months or longer of therapy with Replagal reduces cardiac mass in patients with Fabry Disease.

Study TKT005 demonstrated that, in patients selected for elevated cardiac mass, therapy with Replagal resulted in a statistically significant reduction in cardiac mass as compared with an increase in cardiac mass associated with placebo. In a patient population not selected for elevated cardiac mass (Studies TKT003 and TKT006), statistically significant reductions from baseline in cardiac mass were demonstrated after 12 to 18 months of therapy with Replagal. In female patients, who had markedly elevated cardiac masses at baseline, significant reductions in cardiac mass were seen after six or more months of therapy with Replagal (Study TKT014).

In addition, therapy with Replagal was also associated with significant improvements in cardiac conduction system function as measured by QRS complex duration.

The results of these four studies demonstrate that Replagal reduces cardiac mass consistent with the initiation of the reversal of cardiomyopathy in these patients.

4.5. Additional Effects of Replagal

4.5.1. Metabolic Effects – Increase in Body Weight

The most common result of the gastrointestinal manifestations of Fabry Disease is chronic weight loss **[35,36,37]**. In Study TKT003, there was a statistically significant difference in the changes in weight in the two patient populations. Patients treated with placebo experienced weight loss, but patients treated with Replagal experienced weight gain (p=0.025).

In the Study TKT003, patients initially randomized to placebo had a 1.4 kg decline in their weight during 6 months of placebo therapy (p=0.306, change from Baseline). However, when these patients were treated with Replagal, they experienced a 2.7 kg increase in weight during the first 6 months of therapy (p=0.011, change from Baseline). Weight gain continued during the subsequent 6 months of treatment. At the end of a year of Replagal therapy, these patients experienced a mean weight gain of 4.1 kg (p=0.008, change from Baseline). Thus, coincident with the crossover to Replagal therapy in Study TKT006, the progressive weight loss experienced by these patients was reversed and there was an approximately 9.5% weight gain, which represents a significant metabolic improvement. In the first 6 months of Study TKT011, these patients maintained the weight gain observed in Study TKT006. During the second 6 months of Study TKT011, although a decrease in mean weight was observed compared with the end of Study TKT006, the mean weight of patients remains 2.7 kg greater than at the end of Study TKT003 (p=0.130).

In Study TKT003, patients initially randomized to Replagal, in contrast to the patients treated with placebo, had an approximately 1.4 kg weight gain during Study TKT003, (p=0.043, change from Baseline). During an additional year of treatment in Study TKT006, these patients maintained a net increase in weight of 2.5 kg from the beginning of Study TKT003 (p=0.008, change from Baseline). After 2.5 years of therapy with Replagal, there is a net increase of 1.5 kg in the patients' weight during all 3 studies combined (p=0.207, change from Baseline).

Therapy with Replagal appears to improve the overall metabolism in these patients as represented by these increases in body weight.

4.5.2. Replagal Reverses Hearing Loss

A recent study, by MacDermott and coworkers **[20]**, described sensorineural hearing loss in 78% of male patients with Fabry Disease, and this study defined hearing loss as a significant component of the natural history of Fabry Disease. These data are consistent with the baseline audiogram data from Study TKT005 that also demonstrated sensorineural hearing loss in the patients enrolled in Study TKT005.

An analysis of the effect of Replagal therapy on hearing has been recently presented by Hajioff, *et al*. **[40]**. These preliminary data suggest that long-term therapy with Replagal (18 to 30 months) is associated with improvements in hearing in these patients.

4.5.3. Replagal Improves Cerebrovascular Blood Flow

Exploratory analyses from Studies TKT003 and TKT006 assessing the effect of Replagal on cerebrovascular blood flow in patients with Fabry Disease have been published **[39,41]**. Cerebrovascular blood flow has been measured by both positron emission tomography (PET) and transcranial Doppler ultrasound. In these studies, patients with Fabry Disease have been demonstrated to have abnormal cerebrovascular blood flow. As measured by either PET or transcranial Doppler, Replagal significantly improves cerebrovascular blood flow and velocity compared with placebo. Long-term therapy with Replagal is required to demonstrate the effect as measured by transcranial Doppler (Studies TKT003 and TKT006), but the differences between Replagal and placebo are readily demonstrated by the PET analyses after 6 months of therapy (Study TKT003). These data indicate that Replagal improves cerebrovascular blood flow and velocity in patients with Fabry Disease.

4.6. Efficacy of Replagal: Conclusions

Treatment with Replagal up to 2.5 years has demonstrated the following:

- Improvement of kidney structure and function:
    - Replagal increased the fraction of normal glomeruli compared with a decrease in the fraction of normal glomeruli in patients treated with placebo. In addition, Replagal decreased the fraction of glomeruli with mesangial widening compared to an increase in the fraction of glomeruli with mesangial widening in patients who received placebo.
    - Measurements of standard renal glomerular histopathology correlate with renal function.
    - Long term therapy with Replagal results in improvements in renal function.
    - Replagal may delay the time to progression to ESRD.
- Improvement of cardiac structure and function:
    - Replagal reduces cardiac mass in patients with Fabry Disease.
    - Replagal improves cardiac conduction system function.
- Improvements in metabolic function:
    - Replagal improves metabolic function as manifested by an improvement in patient weight.
    - Replagal decreases Gb_3 levels in plasma and urine sediment (see Section 5).

5. Other Surrogate Markers in Fabry Disease

5.1. Deposition of Gb_3 in the Kidney: Effect of Replagal and Lack of Correlation with Kidney Function

5.1.1. Background

FDA can approve a Biologics Application when a sponsor has demonstrated that the product has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit. As discussed above, Replagal (agalsidase alfa) has been shown to have an effect on the surrogate endpoint of standard glomerular histopathology (as measured by the percentage of normal glomeruli, and the percentage of glomeruli with mesangial widening) that is reasonably likely to predict clinical benefit in Fabry Disease. Another surrogate endpoint has been proposed: clearance of Gb_3 from the interstitial capillary endothelial cells in the kidney, as reported in a study of algasidase beta [**42**] . But because Fabry Disease is not a disease of capillary endothelial cells and in the kidney is not principally an interstital disease, clearance of Gb_3 from interstitial capillary endothelial cells in the kidney is not reasonably likely to predict clinical benefit.

In the discussion in Section 3.2.2 (*Renal Pathology of Fabry Disease*), above, it was noted in Brenner's textbook that the "major site of accumulation of [glycosphingolipid] is the glomerular epithelial cell" **[29]**. This view is confirmed by Desnick *et al*. **[2]**, who states that the lesions of Fabry disease "are due to the accumulation of glycosphingolipids primarily in epithelial cells of the glomerulus and the distal tubules." Desnick *et al* note that the endothelial cells are relatively spared. Alroy *et al* **[43]** have also questioned whether deposition of Gb_3 in the interstitial capillary endothelial cells plays a major role in Fabry disease, stating that "the importance of this parameter as a potential surrogate marker for the progression of renal dysfunction (impaired GFR) and other renal pathology (glomerulosclerosis, interstitial fibrosis) is uncertain and needs to be tested in a longitudinal study."

The following figures show where Gb_3 is - and is not - deposited in Fabry disease. They confirm that Brenner's and Desnick's focus on the epithelial cells as the primary sites of deposition is correct, and also that the endothelial cells are relatively spared.

Figures 18 through 21 are progressively higher magnifications of a toluidine blue stained renal biopsy specimen from a patient with Fabry Disease. Toluidine blue specifically stains Gb_3 dark blue and Gb_3 deposits are seen as blue, round inclusion bodies within cells.

Figure 18: Fabry Kidney: Low Power (Toluidine Blue Stain: 10x)

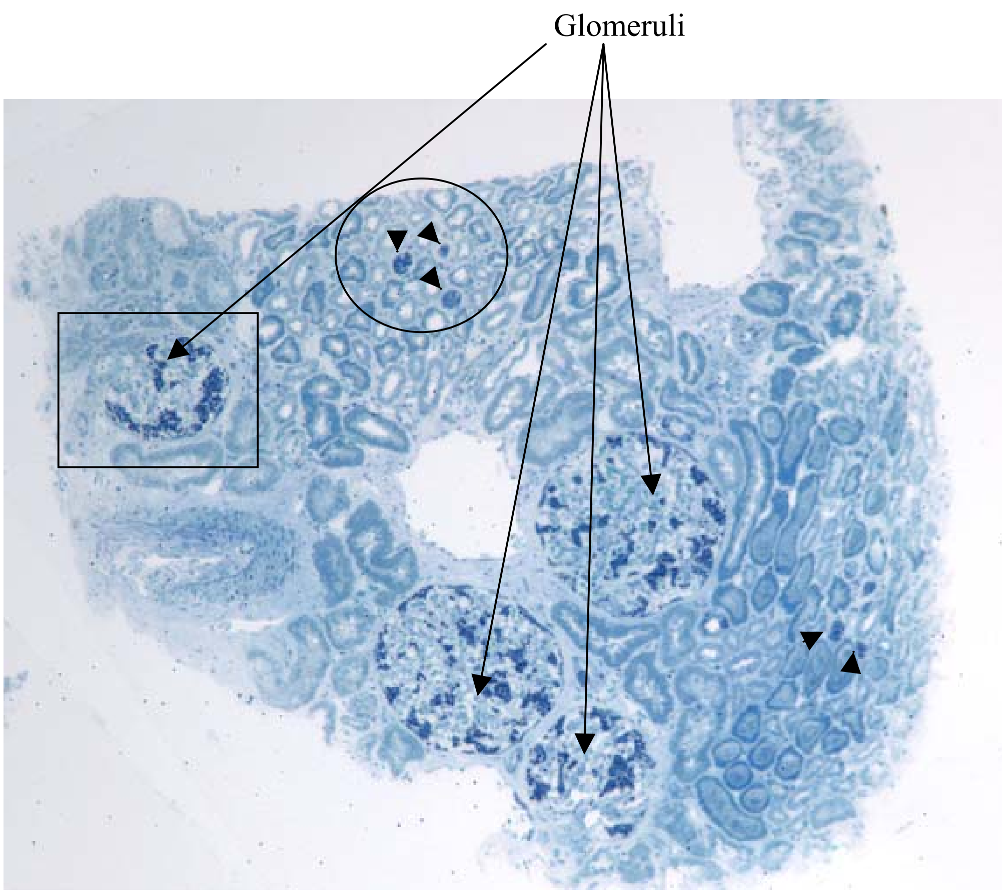
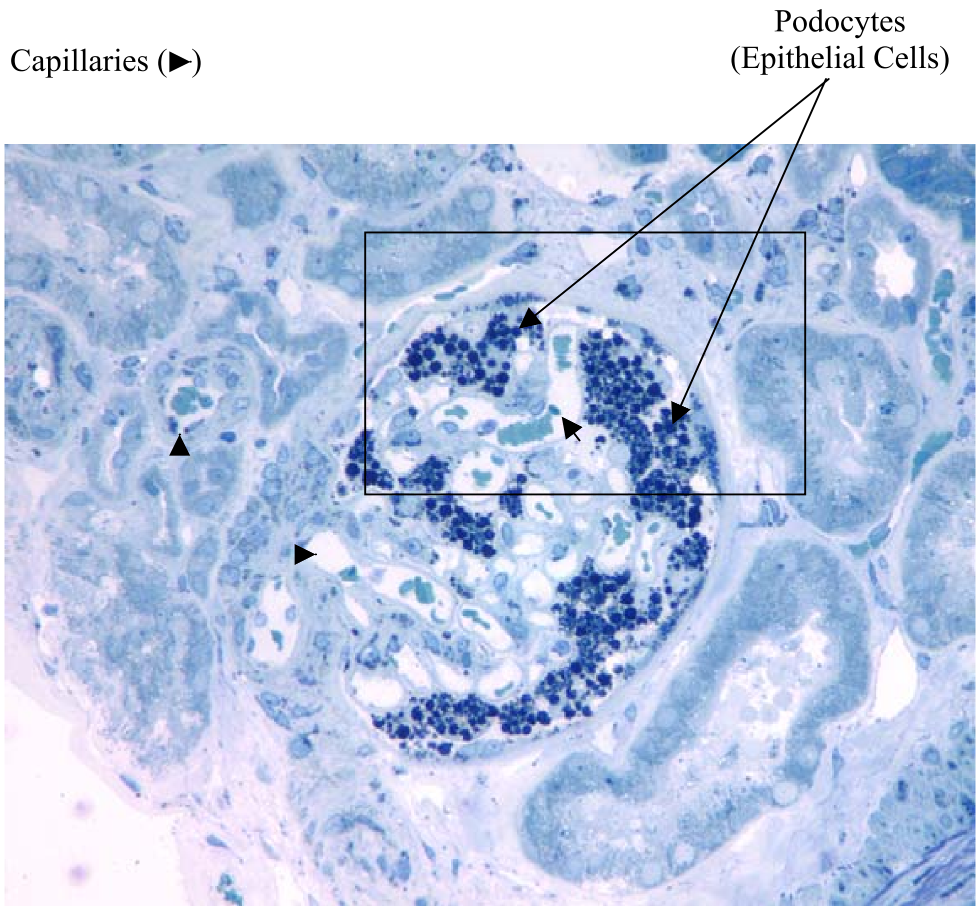


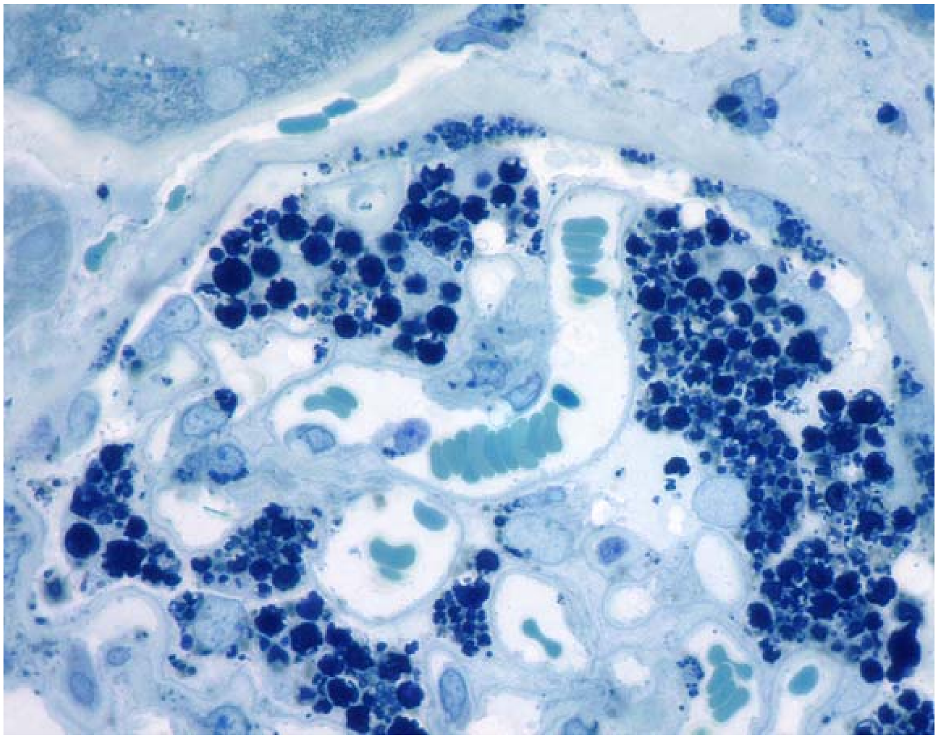
Figure 18 is low power magnification of a kidney patient with Fabry Disease. Multiple glomeruli are seen in this biopsy as noted by the arrows. Also seen (arrow heads) are Gb_3 depositions in tubules. The remainder of the interstitial area is relatively free of glycolipid deposit. As seen at low power, essentially all of the Gb_3 deposition within the kidney is in the glomeruli.

Figure 19: Fabry Kidney: Higher Power (Toluidine Blue Stain: 40x)



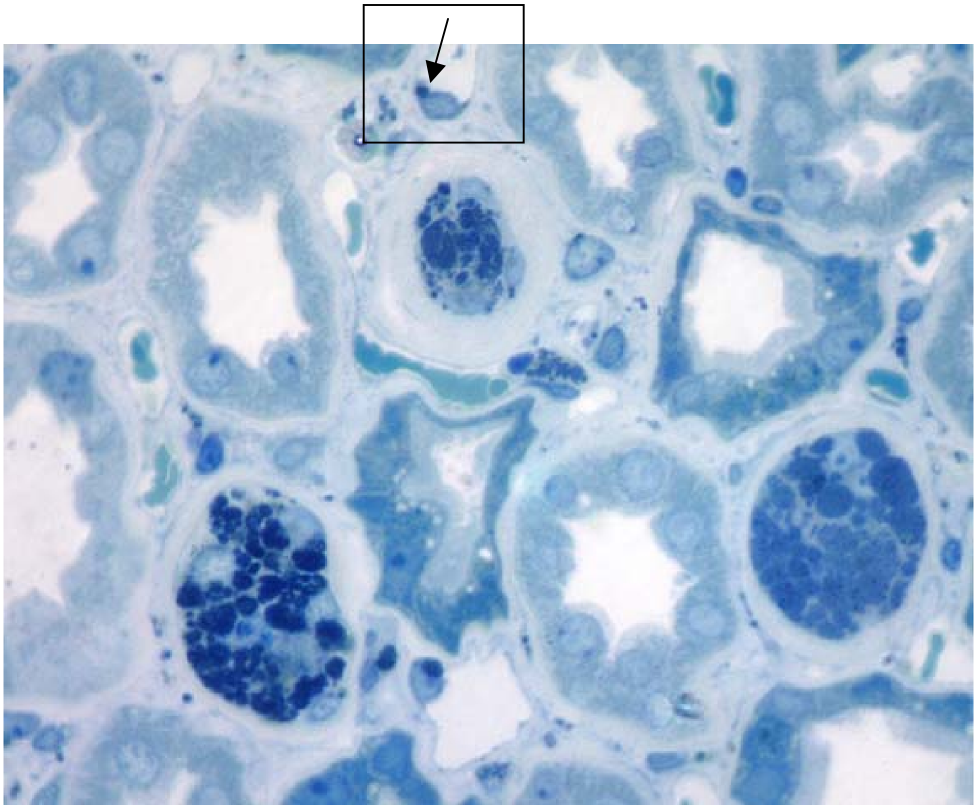
As shown in Figure 19 (a higher power magnification of the boxed glomerulus in Figure 18), the arrows point to podocytes cells that are involved in this glomerulus. As described previously (Section 3.2.2.1), one of the principal features of the kidney pathology of Fabry Disease is renal glomerular epithelial Gb_3 deposition. Of note, multiple renal capillaries, both within the glomerulus and in the interstitium are shown by arrowheads; these capillaries and their corresponding endothelial cells are essentially free of deposited Gb_3. As discussed above, the pathologic hallmarks of endothelial cell disease in the kidney (double contours, swelling of the capillary endothelial, cytoplasm and, and occlusion of capillary lumens) are not seen in Fabry Disease.

Figure 20: Fabry Kidney: High Power (Toluidine BlueStain: 100x)



In Figure 20, a higher power view (100x) of the region of the glomerulus shown in Figure 19 is presented. Again, the epithelial and mesangial nature of the Gb_3 depositions are clearly indicated. In the glomerulus, the capillaries are identified by the presence of red blood cells within their lumens. In each of these capillaries, the endothelial cell has little if any deposited Gb_3. At higher power, the capillaries in the interstitial area are also essentially free of deposited Gb_3.

Figure 21: Fabry Kidney: Tubules (Toluidine Blue Stain; 100x)



Finally, an area of tubular deposition is shown at high power in Figure 21. In Figure 18, this area of tubular deposition is also highlighted with a circle. As shown in Figure 21, there are three swollen renal tubules that are completely occluded by deposited Gb_3 within epithelial cells. On this slide, multiple different interstitial capillaries are shown. The endothelial cells are clearly seen in these capillaries and have very little deposited Gb_3, and the vessels are patent.

At the top of Figure 21, within a box is a small area of Gb_3 deposition within the interstitial capillary endothelial cell.

5.1.2. Effect of Replagal on Gb_3 Deposition in the Kidney

In Study TKT003, the accumulation of Gb_3 in the kidney was assessed in toluidine blue stained kidney biopsy specimens. Deposition of Gb_3 was studied in various cell types, including the interstitial vascular endothelium. Gb_3 deposition was scored on a semi-quantitative scale from 0 to 3, with 0 representing no deposition and 3 representing severe deposition. The results are shown in Table 20.

Table 20: Effect of Replagal on Interstitial Vascular Endothelial Gb_3 Deposition

Vascular Endothelium	Replagal (n=12)	Placebo (n=9)
Baseline	2.0±0.23	1.6±0.29
Change to Week 24	−1.2±0.26	0.2±0.28
p-value	0.003	

As shown in Table 20 there was a significant decrease in interstitial vascular endothelial Gb_3 deposition in patients treated with Replagal compared to an increase in Gb_3 deposition in patients randomized to placebo (p=0.003).

In the study by Eng *et al* **[42],** (also using a 0 to 3 scale) there were similar changes in interstitial capillary endothelial Gb_3 deposition. These results are reproduced in Table 21.

Table 21: Reduction of Interstitial Capillary Endothelial Cell Gb_3 by Agalsidase Beta (Fabrazyme™, Genzyme Corporation)

Interstitial Capillary Endothelial Gb_3 Content	Agalsidase Beta (n=29)	Placebo (n=29)
Baseline	1.9±0.8	2.2±0.7
Change to Week 20	−1.6±1.2	-0.1±1.1
p-value	<0.001	

The effect of Replagal and Fabrazyme on interstitial capillary endothelial cell Gb_3 deposition is similar.

Based on the renal pathology discussion above and the results shown in Tables 20 and 21 the question can be restated: Is Gb_3 deposition in the renal interstitial capillary endothelial cell a good predictor of renal function?

5.1.2.1. Correlation of Renal Function with Vascular Endothelial Cell Gb_3 Deposits in the Kidney

Figures 22A and 22B show the baseline vascular endothelial Gb_3 deposit score from Study TKT003 plotted against GFR and creatinine clearance. There is no correlation between renal vascular endothelial cell Gb_3 deposition and renal function. Interestingly, the four patients with the worst vascular endothelial Gb_3 scores at baseline all had essentially normal renal function.

Figure 22A: Scatter Plot of GFR vs Vascular Endothelial Cell Gb_3

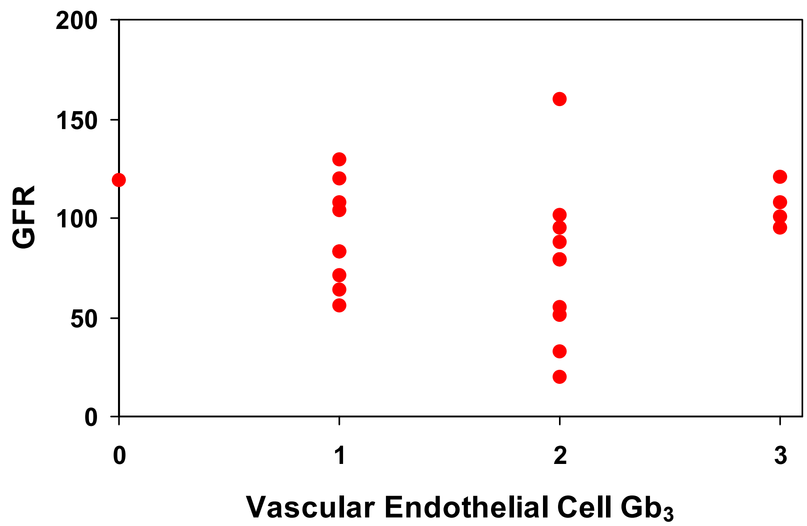
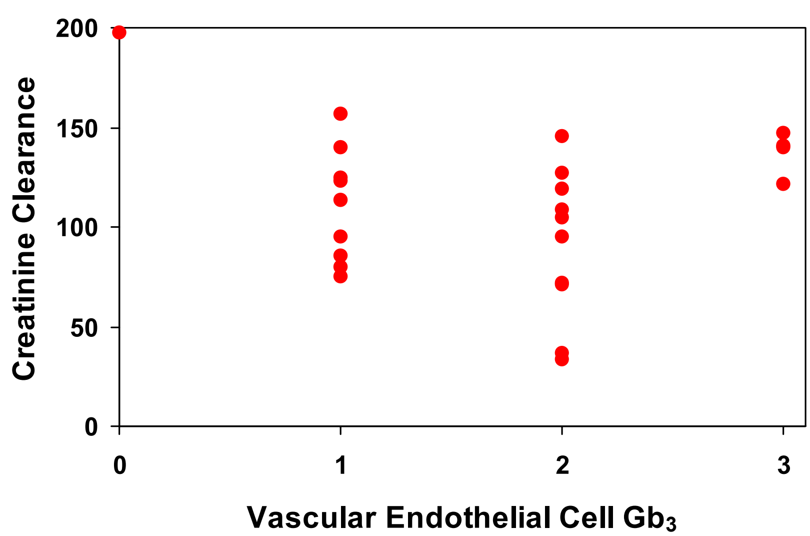


Figure 22B: Scatter Plot of Creatinine Clearance vs Vascular Endothelial Cell Gb_3



5.1.3. Gb_3 Deposition in the Kidney: Summary

Although the involvement of the capillary endothelium is a relatively minor component of Fabry Disease, therapy with Replagal significantly reduces capillary endothelial cell Gb_3 storage. However, as shown above, there is no correlation between the extent of Gb_3 deposition in the capillary endothelial cells and renal function. Since Gb_3 deposition in the capillary endothelial cells cannot be correlated with renal function, it cannot be considered a useful surrogate marker for renal function.

5.2. Plasma Gb_3: Effect of Replagal and Lack of Correlation with Kidney Function

5.2.1. Effect of Replagal on Plasma Gb_3

Plasma Gb_3 has been determined in clinical studies TKT003, TKT006 and TKT011. Table 22 summarizes the data from these long-term dosing studies.

Table 22: Change from Baseline in Plasma Gb_3 Content

Month of Treatment / Study	Mean±SE (nmol/mL)	p-value
Patients Treated with Placebo in TKT003 and Replagal in TKT006 and TKT011 (n=11)		
Month 0 / Baseline of TKT003 (n=11)	10.96±1.087	-
Month 6 / End of TKT003 (n=11)	10.19±1.271	
Month 12 / TKT006 (n=11)	5.98±0.428	
Month 18 / TKT006 (n=11)	5.06±0.675	
Month 24 / TKT011 (n=10)	6.03±0.691	
Month 30 / TKT011 (n=10)	5.57±0.533	
Change - Baseline to End of TKT003 (6 months placebo treatment, n=11)	−0.77±0.479	0.139
Change - End of TKT003 (Month 6) to:		
Month 12 (6 months Replagal treatment, n=11)	−4.21±0.987	0.002
Month 18 (1 year Replagal treatment, n=11)	−5.13±1.238	0.002
Month 24 (1.5 years Replagal treatment, n=10)	−4.10±1.156	0.006
Month 30 (2 years Replagal treatment, n=10)	−4.56±1.147	0.003
Patients Treated with Replagal in TKT003, TKT006, and TKT011 (n=13)		
Month 0 / Baseline of TKT003 (n=13)	12.56±0.867	-
Month 6 / End of TKT003 (n=13)	5.74±0.554	
Month 12 / TKT006 (n=13)	7.59±0.638	
Month 18 / TKT006 (n=13)	6.39±0.578	
Month 24 / TKT011 (n=12)	7.61±0.838	
Month 30 / TKT011 (n=12)	8.19±0.931	
Change - Baseline of TKT003 (Month 0) to:		
Month 6 (6 months Replagal treatment, n=13)	−6.83±0.758	<0.001
Month 12 (1 year Replagal treatment, n=13)	−4.98±0.902	<0.001
Month 18 (1.5 years Replagal treatment, n=13)	−6.17±0.846	<0.001
Month 24 (2 years Replagal treatment, n=12)	−4.87±1.004	0.001
Month 30 (2.5 years Replagal treatment, n=12)	−4.23±0.983	0.001

During the 24 weeks of treatment with placebo in Study TKT003, the patients experienced essentially no change in their plasma Gb_3 levels. Conversely, when treated with Replagal for 6 months in Study TKT006, these patients experienced a marked metabolic correction of plasma Gb_3 levels. Patients experienced an approximately 41% decline in their plasma Gb_3 levels, which was statistically significant ($p=0.002$). This decline was maintained and extended in the subsequent 6 months of treatment. At the end of 1 year, plasma Gb_3 had decreased by approximately 50% ($p=0.002$). In Study TKT011, the metabolic correction observed in Study TKT006 has been maintained; a decrease of approximately 45% in plasma Gb_3 from the end of Study TKT003 to Month 30 (approximately 2 years treatment with Replagal) was observed ($p=0.003$).

The data for the patients treated with Replagal during all 3 studies demonstrate that metabolic correction with Replagal is maintained over 2.5 years. Although a slight increase in plasma Gb_3 levels occurred after the first 6 months of Study TKT006, the net decline in plasma Gb_3 over 2.5 years of treatment with Replagal was approximately 36% ($p=0.001$).

5.2.2. Correlation of Renal Function with Plasma Gb_3

We have attempted to correlate the measurement of plasma Gb_3 levels with renal function in Study TKT003. These data are presented in Figures 23A and 23B.

Figure 23A: GFR vs Plasma Gb_3

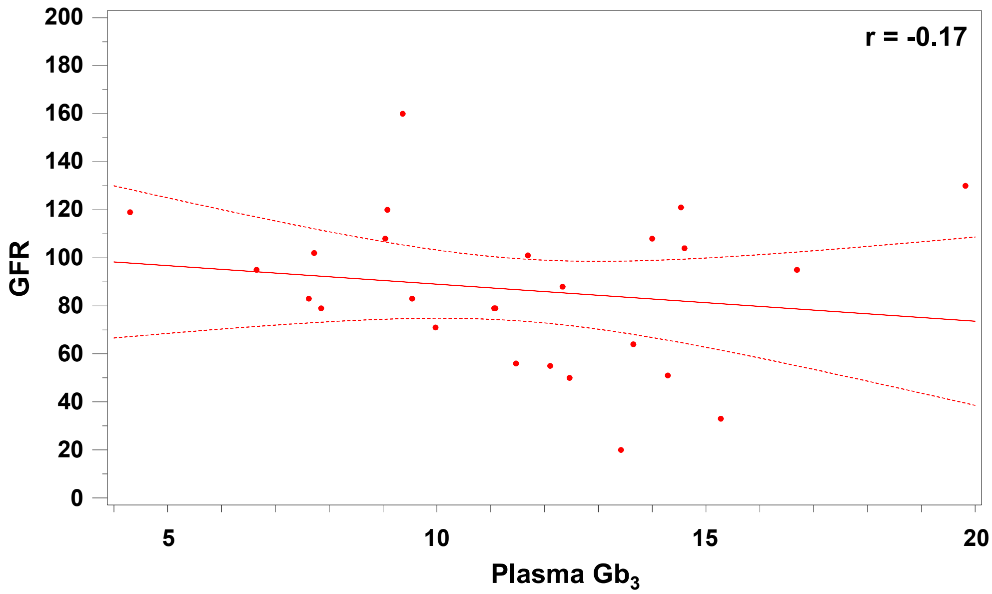
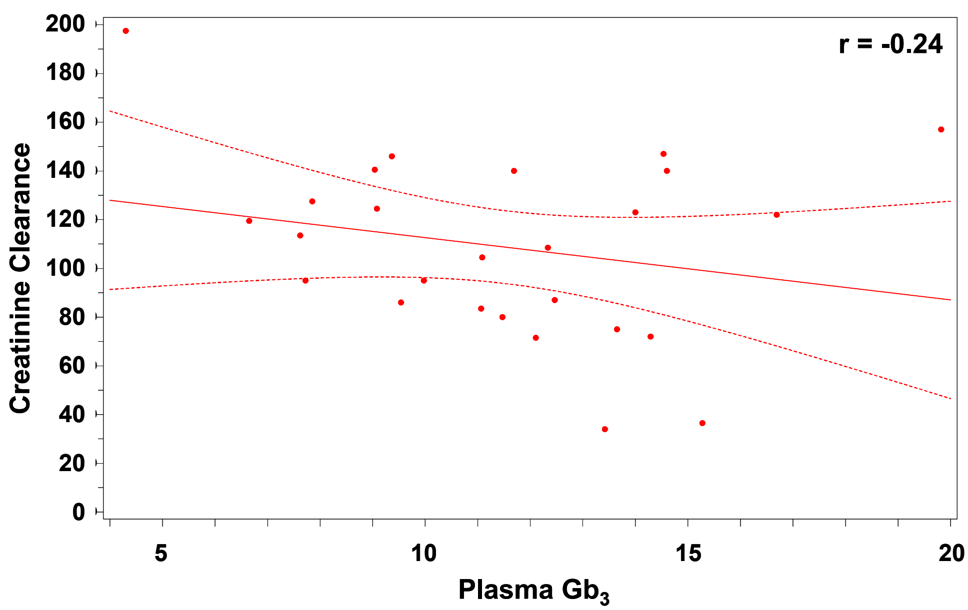


Figure 23B: Creatinine Clearance vs Plasma Gb_3



5.2.3. Plasma Gb_3: Conclusions

As shown in Figures 23A and 23B above, there is no correlation between plasma Gb_3 levels and renal function as measured by GFR (r= -0.17) or creatinine clearance (r= -0.24). Therefore, plasma Gb_3 also cannot be correlated with renal function, and it cannot be considered a useful surrogate marker for renal function.

5.3. Urine Sediment Gb_3: Effect of Replagal and Lack of Correlation with Kidney Function

5.3.1. Effect of Replagal on Urine Sediment Gb_3

Urine sediment Gb_3 has been determined in studies TKT003, TKT006 and TKT011. Table 23 summarizes the data from these studies.

Table 23: Change from Baseline in Urine Sediment Gb_3 Content

Month of Treatment / Study	Mean±SE (nmol/g creatinine)	p-value
Patients Treated with Placebo in TKT003 and Replagal in TKT006 and TKT011 (n=11)		
Month 0 / Baseline of TKT003 (n=11)	2161.8 ± 383.34	
Month 6 / End of TKT003 (n=11)	2494.5 ± 552.67	
Month 12 / TKT006 (n=11)	623.6 ± 124.39	
Month 18 / TKT006 (n=11)	455.7 ± 134.76	
Month 24 / TKT011 (n=10)	596.8 ± 280.17	
Month 30 / TKT011 (n=10)	476.9 ± 189.57	
Change - Baseline to End of TKT003 (6 months placebo treatment, n=11)	332.7 ± 399.77	0.425
Change - End of TKT003 (Month 6) to:		
Month 12 (6 months Replagal treatment, n=11)	-1870.9 ± 505.93	0.004
Month 18 (1 year Replagal treatment, n=11)	-2038.8 ± 496.19	0.002
Month 24 (1.5 years Replagal treatment, n=10)	-1992.2 ± 395.58	0.001
Month 30 (2 years Replagal treatment, n=10)	-2112.1 ± 471.65	0.002
Patients Treated with Replagal in TKT003, TKT006, and TKT011 (n=13)		
Month 0 / Baseline of TKT003 (n=13)	2496.0 ± 303.21	
Month 6 / End of TKT003 (n=13)	1808.6 ± 459.14	
Month 12 / TKT006 (n=13)	1158.8 ± 219.11	
Month 18 / TKT006 (n=13)	867.9 ± 190.92	
Month 24 / TKT011 (n=12)	1493.8 ± 400.66	
Month 30 / TKT011 (n=12)	1322.6 ± 352.17	
Change - Baseline of TKT003 (Month 0) to:		
Month 6 (6 months Replagal treatment, n=13)	-687.4 ± 321.45	0.054
Month 12 (1 year Replagal treatment, n=13)	-1337.2 ± 237.67	<0.001
Month 18 (1.5 years Replagal treatment, n=13)	-1628.1 ± 231.26	<0.001
Month 24 (2 years Replagal treatment, n=12)	-1064.8± 311.89	0.006
Month 30 (2.5 years Replagal treatment, n=12)	-1235.9± 274.16	0.001

During the 24 weeks of treatment with placebo in Study TKT003, the patients experienced minimal change in urine sediment Gb_3 content ($p=0.425$). In contrast, when treated with Replagal for 6 months in Study TKT006, these same patients experienced statistically significant decreases in urine sediment Gb_3 content ($p=0.004$). These patients experienced an approximately 75% decrease in their level of urine sediment Gb_3 in the first 6 months of therapy with Replagal in Study TKT006. Over the entire year of treatment with Replagal, urine sediment Gb_3 decreased by a total of 82% ($p=0.002$). During an additional year of treatment in Study TKT011, patients have maintained a statistically significant decrease of 82% in urine sediment Gb_3 from the end of Study TKT003 ($p=0.002$).

Patients treated with Replagal in all 3 studies demonstrated that the first 6 months of therapy was associated with a marked decline in urine sediment Gb_3 levels. During Study TKT003, patients experienced an approximately 28% decline in their level of urine sediment Gb_3 ($p=0.054$). During the additional year of Study TKT006, these patients experienced a further statistically significant decline in their level of urine sediment Gb_3 ($p=0.013$). Overall, the 18 months of therapy was associated with an approximately 65% decline in urine sediment Gb_3 in these 13 patients, a change that was statistically significant ($p<0.001$). During an additional year of treatment in Study TKT011, although urine sediment Gb_3 increased slightly at Month 24, a net decrease from the beginning of treatment in Study TKT003 (2.5 years of treatment) of approximately 48% has been maintained ($p=0.001$). The mechanism for the rise in urine sediment Gb_3 levels at Months 24 and 30 remains unclear, but may reflect redistribution of body stores and elimination of distant tissue Gb_3 in the urine.

5.3.2. Correlation of Renal Function with Urine Sediment Gb_3

We also have attempted to correlate the measurement of urine sediment Gb_3 levels with renal function in Study TKT003. These data are presented in Figures 24A and 24B.

Figure 24A: GFR vs Urine Sediment Gb_3

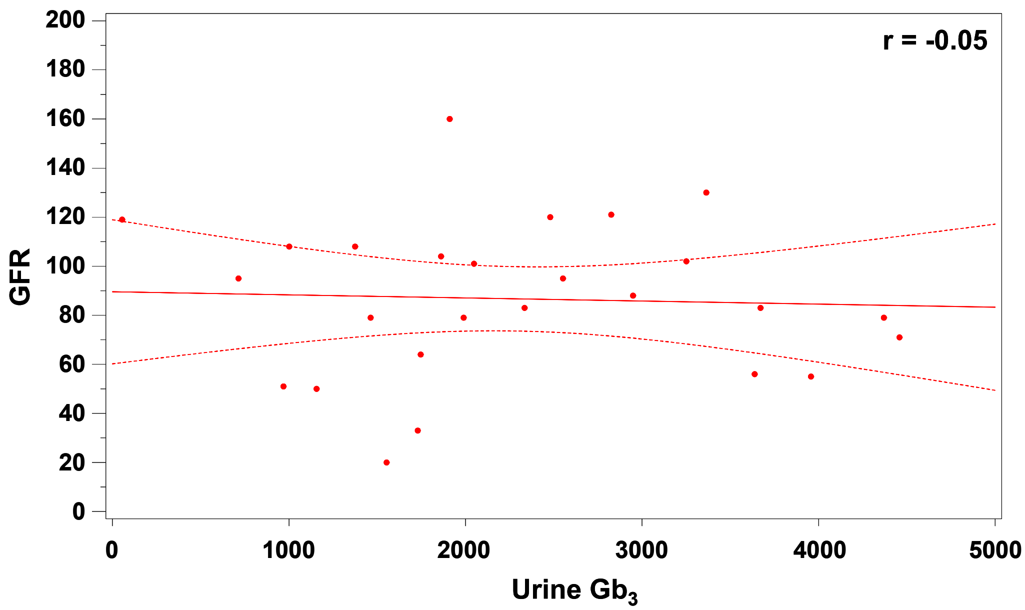
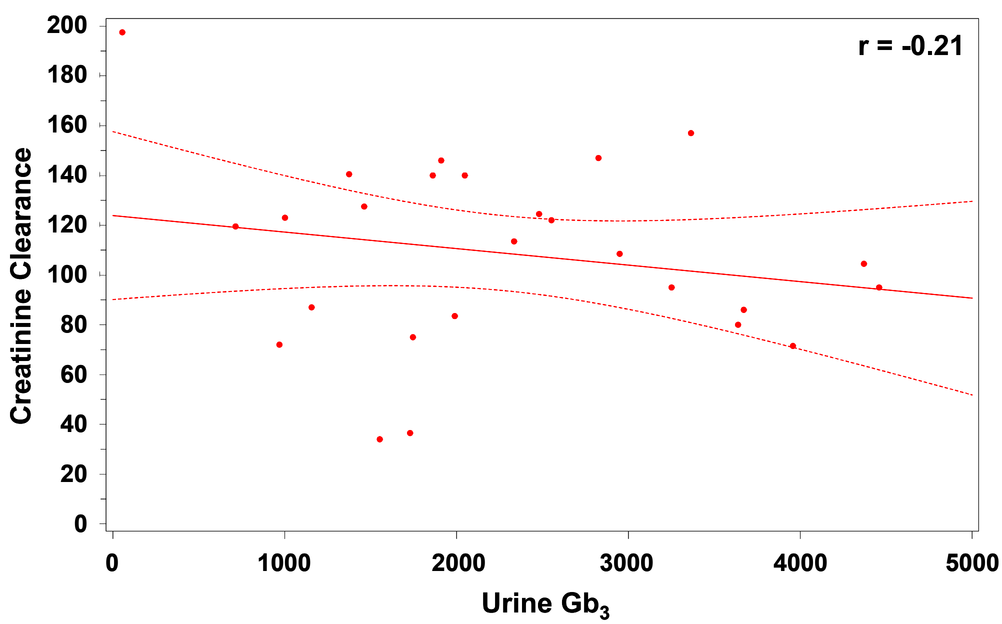


Figure 24B: Creatinine Clearance vs Urine CTH Urine Sediment Gb_3



5.3.3. Urine Gb_3: Conclusions

As shown in Figures 24A and 24B above, there is no correlation between urine sediment Gb_3 levels and renal function as measured by GFR ($r=-0.05$) or creatinine clearance ($r = -0.21$). Therefore, urine sediment Gb_3 also cannot be correlated with renal function, and it cannot be considered a useful surrogate marker for renal function.

5.4. Conclusions

Measurements of standard renal glomerular histopathology correlate with clinical measures of renal function (both GFR and creatinine clearance). Therefore, the measurement of standard renal glomerular histopathology represents a valid surrogate marker of clinical efficacy in Fabry Disease. Measurements of standard renal glomerular histopathology are reasonably likely to predict clinical benefit.

Measurements of Gb_3 content in capillary endothelial cells in the kidney, plasma, and urine sediment do not correlate with measurements of renal function. Therefore, measurements of Gb_3 do not appear to be useful surrogate markers for the efficacy of enzyme replacement therapy in Fabry Disease.

6. SUMMARY OF SAFETY RESULTS

6.1. Overview

To date, over 300 patients have received at least one dose of Replagal in clinical trials, compassionate use, and commercial use (Note: Replagal was approved by the European Commission in August 2001). The safety database now represents approximately 10% of the predicted Fabry Disease population in the United States, European Union and Canada. Within the subset of patients who have received Replagal in clinical trials, 76 patients have received drug for a duration ranging from 2 weeks (one dose) to >36 months. Approximately one-third of the patients have received Replagal for 0-6 months, one-third for 6-30 months, and one-third for greater than 30 months. This is represented in the Table 24 below.

Table 24: Duration of Exposure of Patients to Replagal in Clinical Trials

Duration of Exposure to Replagal (months)	Total Number of Patients	Percent
0-6 months	25	32.9%
6-12 months	11	14.5%
12-18 months	5	6.6%
18-24 months	2	2.6%
24-30 months	8	10.5%
30-36 months	13	17.1%
≥36 months	12	15.8%
Total (any duration)	76	100%

The safety data discussed below will focus on clinical trials (TKT001, TKT003, TKT005, TKT006, TKT011, TKT014). The safety data received to date from compassionate use and commercial use are consistent with clinical trial safety data discussed below.

6.2. Non-Serious Adverse Events

Adverse Events from Placebo-Controlled Clinical Trials

In placebo-controlled trials, patients in the Replagal group had a similar number of adverse events as patients in the placebo group for most body systems. The most commonly reported adverse events in the Replagal treated groups included: headache, influenza-like symptoms, back pain, paresthesia, pain, rigors, fever, diarrhea, nausea, allergic reaction and pharyngitis. Events that were reported at a frequency greater than 10% higher in the Replagal group compared with placebo included allergic reaction, fever, influenza-like symptoms, rigors, dizziness, paresthesia, diarrhea, and skeletal pain. Events that were reported at a frequency greater than 10% higher patients in the placebo group compared with the Replagal treated group included: back pain, dyspnea, rash, albuminuria and abnormal renal function. The majority of adverse events were mild-to-moderate in severity, and represent symptoms typically observed as part of the natural history of Fabry Disease. In addition, data from study TKT014 indicate that the safety profile of Replagal is excellent in female patients with Fabry Disease, and is similar to that observed in previous studies of Replagal in male patients. Appendix 1 lists any adverse events that occurred at a frequency of >20% in either the Replagal or placebo treated groups for both Studies TKT003 and TKT005.

Note: Analysis of safety data from TKT010 is ongoing.

Adverse Drug Reactions from Clinical Trials

Table 25 below lists those adverse drug reactions (ADRs) reported for patients treated with agalsidase alfa in clinical trials, where causality is at least suspected in one or more cases. Information is presented by system organ class and frequency (common >1/100; very common >1/10). The occurrence of an event in a single patient is defined as common in view of the number of patients treated. A single patient could be affected by several ADRs.

Table 25: List of Common and Very Common Adverse Drug Reactions from Clinical Trials of Replagal

Body System Disorders	Very Common (>1/10)	Common (>1/100)
Psychiatric		insomnia, agitation
Nervous System	neuralgia, headache	dizziness, tremor, paresthesia, dysesthesia, dysphonia, hypoesthesia, vertigo
Visual System		vision disorder, lacrimation abnormal
Special Senses		parosmia, taste perversion
Cardiovascular		hypertension, tachycardia, T-wave inversion
Vascular	flushing	vascular disorder
Respiratory		throat tightness, respiratory insufficiency, hypoxia, dyspnea, coughing, laryngitis, snoring
Gastrointestinal	nausea	abdominal pain, diarrhea, vomiting
Skin and Appendages	erythematous rash, acne	rash, pruritus, dry skin, skin disorder
Metabolic		periorbital edema
Musculoskeletal		arthralgia, myalgia, skeletal pain
Body As a Whole	infusion related effects, chest pain, rigors, fever, fatigue, back pain, heat intolerance, leg pain	pain, influenza-like symptoms, edema, peripheral edema, temperature sensation changed

6.3. Deaths

There have been a total of eight reports of death among the greater than 300 patients who have received Replagal in clinical trials, compassionate use protocols or on a commercial basis. None of the deaths were assessed as related to study drug or Replagal.

A 38-year-old male died suddenly of post-operative complications following renal transplantation.

A 56-year-old female patient with advanced Fabry Disease, including renal insufficiency, cardiomyopathy, and intermittent atrial fibrillation, died from a cerebral embolism following a myocardial infarction.

A 54-year-old male patient with a history of aortic insufficiency, irregular heart rate, mitral valve disease, left ventricular hypertrophy, chronic atrial fibrillation, and acute renal failure, died after surgery for toxic megacolon, which had developed after the patient developed severe Clostridium difficile colitis. During surgery, the patient experienced a myocardial infarction, adult respiratory distress syndrome, and disseminated intravascular coagulation.

A 53-year-old female with a history of ESRD and cardiomyopathy died of an arrthythmia.

A 53-year-old male with ESRD elected to discontinue dialysis and died. Three months prior to discontinuation of dialysis the patient discontinued Replagal.

A 48-year-old male was hospitalized for pneumonia and subsequently died of ventricular fibrilation.

A 69-year-old male with a history of chronic renal failure died of ESRD.

A 45-year-old male with a history of severe Fabry related hearing loss died of ventricular fibrilation following surgery for insertion of a cochlear implant.

6.4. Withdrawals Due to Adverse Events

There have been no withdrawals from studies TKT001, TKT003, TKT005, TKT006, TKT011, and TKT014 due to adverse events.

6.5. Serious Adverse Events (SAEs)

A variety of serious adverse events have been observed in these Replagal clinical trials. The investigator has assessed three of these SAEs (two allergic reactions and one fever) as possibly or probably related to Replagal. The majority of events observed are consistent with commonly reported symptoms in untreated Fabry Disease. These include events in the major organ systems that account for the morbidity and mortality in Fabry Disease, such as: renal (eg, renal failure, fluid overload), cardiac (eg, myocardial infarction, coronary artery disease), and cerebrovascular (eg, transient ischemic attack, cerebral infarction). A table of all SAE's by study number, patient number and reported term, is included in Appendix 2. Each reported term describes a separate SAE for that particular patient in the table.

6.6. Infusion Reactions

In approximately 10% of patients, Replagal has been associated with mild, acute idiosyncratic infusion reactions, during or within one hour following infusion. The most common symptoms have been chills and facial flushing. Such reactions have generally occurred within the first 2-4 months after initiation of treatment with Replagal. The infusion can be temporarily interrupted (5 to 10 minutes) until symptoms subside and the infusion may then be restarted. Mild and transient effects generally do not require medical treatment or discontinuation of the infusion. In addition pre-treatment, generally with oral antihistamines and/or corticosteroids, from 1 to 3 hours prior to infusion has prevented subsequent reactions in those cases where prophylaxis was indicated.

Infusion reactions were initially observed in Replagal treated patients at a frequency of 57% (8 of 14 patients) in TKT003. Subsequently, the infusion time was increased from 20 minutes to 40 minutes, resulting in a dramatic decrease in the incidence of infusion reactions. The combined incidence of infusion reactions is approximately 10%. In addition, in TKT014, an open-label study of Replagal in females with Fabry Disease, no infusion reactions occurred.

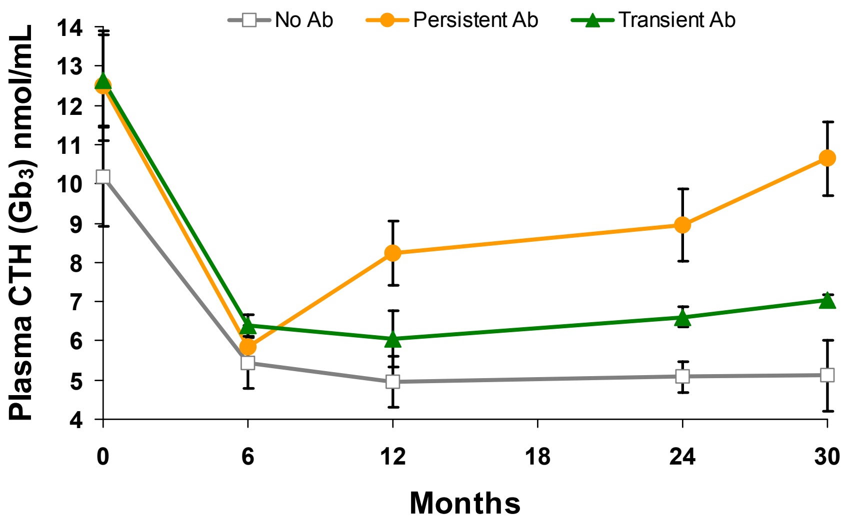
6.7. Antibodies to agalsidase alfa

6.7.1. IgG Response

Approximately 50% of patients who received Replagal in multidose studies developed low titer IgG antibodies. Among 40 male patients followed for up to 2.5 years in clinical studies, persistent IgG antibodies were observed in 11 patients (28%). Most patients were positive at 1:50 or 1:100 dilutions, with 1 patient positive at a 1:2,500 dilution. No patients were positive for IgE, IgA, or IgM antibodies. Serial antibody measurements using several different assay methods indicate that many patients with antibodies develop immunological tolerance to agalsidase alfa, as shown by the disappearance or decrease in antibody titers over time. In study TKT014, none of the female patients treated with Replagal for 3 to 12 months developed antibodies.

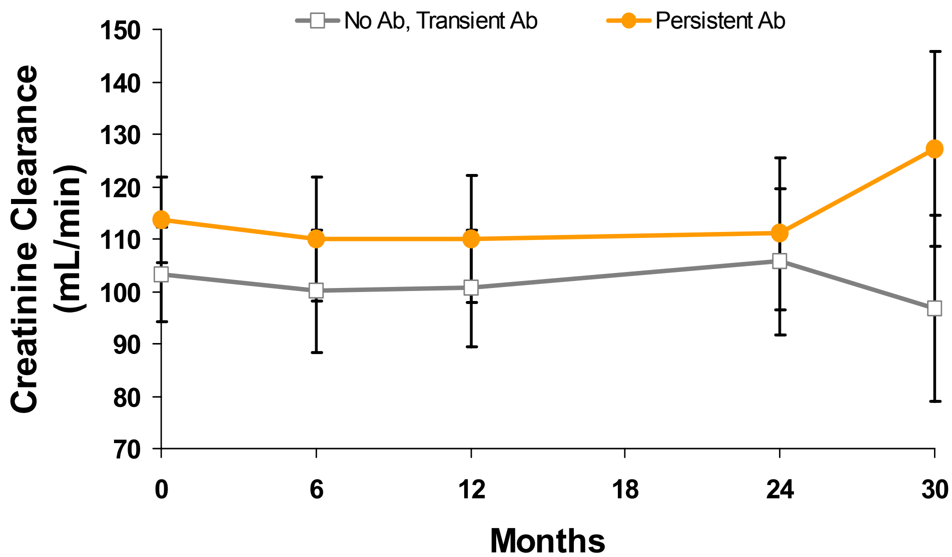
Among patients originally enrolled in Study TKT003, 9/25 patients (36%) were IgG positive after 6 months of treatment. Of the 22 patients that completed month 30 of Study TKT011, 8 remain IgG positive by ELISA. As shown in Figure 25, patients that did not show an antibody response maintained substantially lowered plasma Gb_3 levels over time.

Figure 25: Plasma Gb3 – Subjects Completing Study TKT011



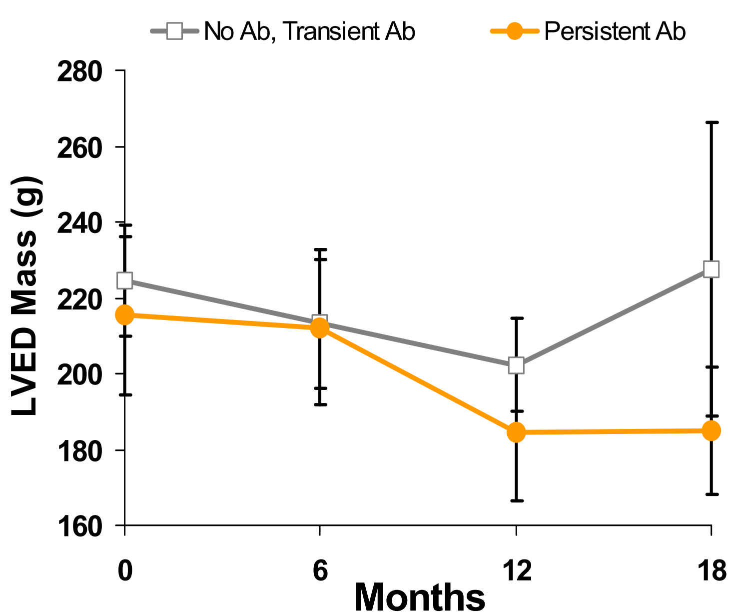
The 8 patients that showed persistent antibodies, however, had a gradual increase in plasma Gb_3 levels over time after an initial reduction. Three patients with transient antibodies showed a small increase in plasma Gb_3 levels over time. Similar correlations were seen when urine sediment Gb_3 was monitored over time in these patient subsets. In general, the presence of IgG antibodies had an effect on pharmacokinetics, with antibody positive patients showing generally increased clearance of Replagal. However, there is no evidence that the presence of persistent antibodies had any clinically significant effects on safety or efficacy. As shown in Figure 26, patients with persistent antibodies in the TKT003/006/011 study series maintained and ultimately improved their kidney function as measured by creatinine clearance.

Figure 26: Creatinine Clearance – Patients Completing TKT011



In addition, as shown in Figure 27, patients with persistent antibodies in the TKT003/006/011 studies did not differ from antibody negative patients and patients with transient antibodies with regard to their improvement in left ventricular mass.

Figure 27: LVED Mass – Patients Completing TKT011



6.7.2. IgE Antibody Response

No patients treated with Replagal have developed an IgE antibody response. There have been no adverse events observed that would be consistent with an IgE mediated syndrome.

6.7.3. Immune Complexes

Development of antibodies to agalsidase alfa has not been associated with the activation of complement. In addition, there have been no adverse events to suggest immune complex deposition disease. The electronmicrographs of the kidney performed at the end of Study TKT003 were reviewed for the presence of immune complex deposition and there was no evidence of immune complex deposition in any patient in Study TKT003.

6.7.4. Antibodies to agalsidase alfa: Conclusions

Persistent antibodies to agalsidase alfa may appear in approximately 30% of patients. Although the mean reductions in plasma and urine sediment Gb_3 are lower for the subset of patients with persistent antibodies, Gb_3 levels remain below baseline after 2-2.5 years of therapy and there is no apparent effect of IgG antibodies on renal function as measured by creatinine clearance or cardiac mass, as measured by MRI. No patients treated with Replagal have developed an IgE antibody response and there have been no adverse events observed that would be consistent with an IgE mediated syndrome. Overall, there is no apparent correlation between IgG status and the incidence of infusion reactions and there is no evidence of immune complex deposition in the kidney. Although IgG antibodies do occur in a significant fraction of male patients treated with Replagal, the effects on safety and efficacy appear to be minimal.

6.8. Safety Data: Conclusions

- Long-term safety experience for patients treated for up to 2.5 years demonstrates that Replagal has an excellent safety profile.

- The incidence of infusion reactions with the recommended 40-minute infusion rate is approximately 10%, and these events are mild and generally self-limited. In a subset of patients who have infusion reactions, these reactions are controlled with a simple pre-medication regimen of oral antihistamines and/or oral corticosteriods. Thus, greater than 90% of patients who receive Replagal do not require the use of routine pre-medications.

- Low titer IgG antibodies develop in approximately 50% of male patients and zero of 15 female patients treated in Study TKT014. These low titer IgG antibodies to agalsidase alfa are not associated with any clinically significant effects on safety or efficacy. Most of the antibody positive patients showed evidence of tolerance based on a reduction in antibody levels in one or more assays over time.

- No patients treated with Replagal have developed an IgE antibody response. There have been no adverse events observed that would be consistent with an IgE mediated syndrome.

- There is no evidence for immune complexes in antibody positive patients who continue to receive therapy with Replagal.

7. Overall Risk-Benefit Summary

Therapy with Replagal has been demonstrated to be both safe and effective for the treatment of patients with Fabry Disease.

Clinical studies with Replagal have demonstrated the following.

- Improvement of kidney structure and function:
    - Replagal increased the fraction of normal glomeruli compared with a decrease in the fraction of normal glomeruli in patients treated with placebo. In addition, Replagal decreased the fraction of glomeruli with mesangial widening compared to an increase in the fraction of glomeruli with mesangial widening in patients who received placebo.
    - Measurements of standard renal glomerular histopathology correlate with renal function.
    - Long term therapy with Replagal results in improvements in renal function.
    - Replagal may delay the time to progression to ESRD.
- Improvement of cardiac structure and function:
    - Replagal reduces cardiac mass in patients with Fabry Disease.
    - Replagal improves cardiac conduction system function.
- Improvements in metabolic function
    - Replagal improves metabolic function as manifested by an improvement in patient weight.
    - Replagal decreases Gb_3 levels in plasma and urine sediment.
- Replagal is safe and well-tolerated.

APPENDIX 1

Adverse Events Observed in >20% of Patients in Studies TKT003 and TKT005

Body System Preferred Term	**Replagal (n = 21) N (%)**	**Placebo (n = 20) N (%)**
Body As A Whole		
Allergic Reaction	8 (38%)	3 (15%)
Back Pain	11 (52%)	13 (65%)
Chest Pain	7 (33%)	5 (25%)
Fever	8 (38%)	4 (20%)
Influenza-like symptoms	12 (57%)	9 (45%)
Peripheral Edema	6 (29%)	6 (30%)
Pain	9 (43%)	10 (50%)
Rigors	9 (43%)	2 (10%)
Central and Peripheral Nervous System		
Dizziness	7 (33%)	4 (20%)
Headache	17 (81%)	15 (75%)
Paresthesia	10 (48%)	7 (35%)
Gastrointestinal System		
Abdominal Pain	7 (33%)	8 (40%)
Diarrhea	8 (38%)	5 (25%)
Nausea	8 (38%)	9 (45%)
Vomiting	7 (33%)	6 (30%)
Musculoskeletal System		
Arthralgia	5 (24%)	4 (20%)
Skeletal Pain	7 (33%)	2 (10%)
Respiratory System		
Coughing	7 (33%)	6 (30%)
Dyspnea	0 (0%)	6 (30%)
Pharyngitis	8 (38%)	7 (35%)

Adverse Events Observed in >20% of Patients in Studies TKT003 and TKT005

Body System Preferred Term	**Replagal (n = 21) N (%)**	**Placebo (n = 20) N (%)**
Skin and Appendages		
Rash	2 (9%)	5 (25%)
Urinary System		
Albuminuria	3 (14%)	6 (30%)
Renal Function Abnormal	2 (9%)	5 (25%)
Infection	8 (38%)	6 (30%)

APPENDIX 2

Table of Serious Adverse Events in Clinical Trials with Replagal

Study Number	Patient Number	As Reported or Preferred Term
TKT001	0001	Fever (following a dental procedure)
TKT003	0016	Injection site bleeding; Allergic Reaction*; Chest Pain
	0018	Allergic Reaction*
	0021	Hearing Decreased
	0027	Anemia; Fever*
TKT005	0009	Pharyngitis
TKT006	0001	Ataxia; Chest Pain
	0005	Hearing Decreased
	0007	Hearing Decreased
	0010	Viral Hepatitis
	0012	Abdominal Pain
	0014	Coronary Artery Disorder; Burn
	0016	Vitamin B12 Deficiency; Muscle Weakness
	0021	Cerebrovascular Disorder
TKT011	0003	Cerebral Infarction
	0005	Urinary Tract Infection Chronic Renal Failure
	0006	Pneumonia; Bradycardia
	0010	Abdominal Pain
	0011	Transient Ischemic Attack; Cellulitis (3 events)
	0014	Acute prostatitis; Pneumonia; Bladder Dysfunction; Chest Pain; Paroxysmal Atrial Fibrillation; Mood Disorder; Myocardial Infarction
	0026	Cerebellar Infarction; Transient Ischemic Attack; Coronary Disorder; Coronary Artery Occlusion
TKT014	0001	Hypertension/Dizziness
	0002	Acute Exacerbation of COPD; Acute Cerebral and Mesangial Embolus; Myocardial Infarction
	0011	Cerebrovascular Accident; Acute Hearing Loss

* Probably or possibly related to Replagal

APPENDIX 3

RENAL PATHOLOGY IN STUDY TKT003

In Study TKT003, renal biopsies were obtained from patients at baseline and at week 24. Using the percutaneous approach, the goal was to obtain two renal cortical tissue samples ("cores") of approximately 1-2 cm in length. Following biopsy, the two cores were divided into thirds:

- The middle one-third of each core was used for Gb_3 measurement.

- The inner one-third of the first core and the outer one-third of the second core were processed for light microscopy (formalin fixation and paraffin embedding).

- The outer one-third of the first core and the inner one-third of the second core were processed for electron microscopy (glutaraldehye fixation and plastic embedding).

PROCESSING OF RENAL BIOPSY SAMPLES

The cores (samples) were embedded in paraffin or plastic blocks at the Armed Forces Institute of Pathology using AFIP standard operating procedures. AFIP's Histopathology Laboratory is certified by the College of American Pathologists. The samples from each subject were considered as one biopsy, were coded as one, and were processed together.

Once the blocks of tissue had been prepared at AFIP, they were sent in batches to TKT, where TKT personnel assigned a random number to each set of blocks corresponding to a given biopsy (ie., baseline or week 24). Coded blocks were returned in batches to AFIP for sectioning and staining for light microscopy. AFIP standard operating procedures were used. To assure consistency of sectioning and staining, the baseline and week 24 biopsies for each subject were always included in the same batch. Since they were assigned random numbers, the baseline and week 24 biopsies could not be paired.

Three slides were prepared (one each stained by H & E, PAS, and Masson) for each biopsy specimen obtained at Baseline or Week 24. In most cases, two specimens were obtained per biopsy procedure resulting in 6 slides (two H&E, two PAS, and two Masson) being examined. Because of the preparation of multiple plastic tissue blocks per biopsy, up to 6 toluidine blue slides were also examined. Therefore, the numbers and types of slides submitted varied with the amount of kidney tissue obtained, but, in general, were the following:

- Two H&E stained slides
- Two PAS stained slides
- Two Masson stained slides
- Three to six toluidine blue stained slides

Each slide from a given biopsy bore the same random number code.

SCORING

Two AFIP pathologists, Sharda Sabnis, MD and Ashwini Chavan, MD, read and scored the slides. They were blinded to patient identification, treatment assignment (Replagal or placebo), and order of biopsy (baseline or week 24 biopsy).

Active Lipid Damage Score

The definition of the ALDS was the combined sum of the six individual elements shown in Table 3-1. The individual elements were graded on a scale of 0 to3 (0=normal, 1=mild, 2=moderate, 3=severe and diffuse). Thus, the ALDS total score had a range of 0 to 18. Toluidine blue-stained slides, which readily demonstrate Gb_3 deposits, were used to score the components of the ALDS.

Table 3-1 presents the ALDS analysis as well as the analyses of each of the individual six components. The change from Baseline for each of the individual components was assessed with ANCOVA; however, assessment of the individual components as presented in Table 3-1 was not a prespecified analysis.

Table 3-1: ALDS and its components

	Replagal	Placebo
Acute Lipid Damage Score		
Baseline	9.4 ± 0.61	8.3 ± 1.20
Change to Week 24	–1.5 ± 0.81	0.9 ± 0.93
p-value	0.114*	
Glomerular Epithelial Cells		
Baseline	2.3 ± 0.21	2.4 ± 0.26
Change to Week 24	0.0 ± 0.30	0.0 ± 0.27
p-value	0.827†	
Endocapillary Cells		
Baseline	1.2 ± 0.13	1.1 ± 0.23
Change to Week 24	-0.7 ± 0.21	0.0 ± 0.27
p-value	0.037†	
Proximal Tubules		
Baseline	0.1 ± 0.09	0.0 ± 0.0
Change to Week 24	-0.1 ± 0.09	0.1 ± 0.11
p-value	0.305†	
Distal Tubules		
Baseline	1.8 ± 0.23	1.9 ± 0.35
Change to Week 24	0.2 ± 0.38	0.0 ± 0.17
p-value	0.724†	
Vascular Endothelium		
Baseline	2.0 ± 0.23	1.6 ± 0.29
Change to Week 24	–1.2 ± 0.26	0.2 ± 0.28
p-value	0.003†	
Vascular Media		
Baseline	2.3 ± 0.19	1.8 ± 0.32
Change to Week 24	0.0 ± 0.23	0.2 ± 0.36
p-value	0.800†	

* The prospectively defined analysis

† All analyses of the individual components used the same analytical method (ANCOVA) as the total score, but all of the individual analyses are post-hoc analyses.

Chronic Damage Score

The analysis of the individual components of the CDS that were collected and the CDS itself are presented in Table 3-2 below.

Table 3-2: CDS and its components

	Replagal	Placebo
Chronic Damage Score		
Baseline	7.0 ± 0.91	7.0 ± 1.57
Change to Week 24	0.1 ± 0.72	0.4 ± 1.57
p-value	0.808*	
Tubular Atrophy		
Baseline	0.9 ± 0.28	0.8 ± 0.32
Change to Week 24	–0.2 ± 0.18	0.1 ± 0.26
p-value	0.371†	
Interstitial Inflamation		
Baseline	0.6 ± 0.24	1.0 ± 0.37
Change to Week 24	0.1 ± 0.21	–0.1 ± 0.20
p-value	0.775†	
Interstitial Fibrosis		
Baseline	1.0 ± 0.27	1.0 ± 0.33
Change to Week 24	0.0 ± 0.19	0.2 ± 0.28
p-value	0.435†	
Arterial Hyalinosis		
Baseline	0.8 ± 0.17	0.8 ± 0.22
Change to Week 24	–0.4 ± 0.23	0.0 ± 0.33
p-value	0.300†	
Arterial Medial Thickening		
Baseline	1.3 ± 0.26	1.1 ± 0.42
Change to Week 24	0.3 ± 0.22	–0.1 ± 0.54
p-value	0.298†	
Arteriolar Hyalinosis		
Baseline	0.9 ± 0.23	1.1 ± 0.31
Change to Week 24	–0.1 ± 0.15	0.1 ± 0.45
p-value	0.461†	
Arteriolar Medial Thickening		
Baseline	1.6 ± 0.19	1.2 ± 0.32
Change to Week 24	–0.1 ± 0.19	0.2 ± 0.40
p-value	0.735†	

* The prospectively defined analysis

† All analyses of the individual components used the same analytical method (ANCOVA) as the total score, but all of the individual analyses are *post hoc* analyses.

Standard Histopathology

In addition to reviewing the slides using the ALDS and CDS scoring systems, the reviewing pathologists also scored the slides using measures of standard histopathology. They developed a scoring system and put it in writing. Although standard histopathology was not included in TKT's statistical analysis plan, the scoring system was defined while the pathologists were blinded and before they scored the slides. In that sense, it was prospectively established.

The definitions for the components of the Standard Histopathology Analysis were:

- Normal glomeruli were defined as those without an increase in the mesangial matrix
- Mesangial widening was defined as diffuse mesangial matrix increases or widening
- Segmental sclerosis was defined as a focal area of solidification (the focal segmental glomerulosclerosis or FSGS lesion)
- Obsolescence was defined as global sclerosis or complete sclerosis of a glomerulus.

The results of the standard histopathology analysis are shown in Tables 3-3, 3-4, 3-5, and 3-6.

Table 3-3. Kidney Pathology - Normal Glomeruli

Kidney Pathology - Fraction of Normal Glomeruli	Replagal (n=12)	Placebo (n=9)
Baseline – Mean ± SE	0.399 ± 0.066	0.596 ± 0.068
Week 24 – Mean ± SE	0.480 ± 0.089	0.436 ± 0.101
Change from Baseline - Week 24		
Mean±SE	0.082 ± 0.044	–0.159 ± 0.076
% Change	21%	-27%
p-value	0.012	

Table 3-4. Kidney Pathology - Fraction of Glomeruli with Mesangial Widening

Kidney Pathology - Mesangial Widening	Replagal (n=12)	Placebo (n=9)
Baseline – Mean ± SE	0.382 ± 0.043	0.239 ± 0.038
Week 24 – Mean ± SE	0.257 ± 0.060	0.404 ± 0.095
Change from Baseline - Week 24		
Mean ± SE	–0.125 ± 0.050	0.165 ± 0.077
% Change	–33%	69%
p-value	0.010	

Table 3-5. Kidney Pathology - Fraction of Glomeruli with Segmental Sclerosis

Kidney Pathology - Segmental Sclerosis	Replagal (n=12)	Placebo (n=9)
Baseline – Mean ± SE	0.028 ± 0.014	0.060 ± 0.018
Week 24 – Mean ± SE	0.068 ± 0.025	0.030 ± 0.019
Change from Baseline - Week 24 Mean ± SE	0.040 ± 0.021	–0.031 ± 0.016
p-value	0.048	

Table 3-6. Kidney Pathology - Fraction of Glomeruli with Obsolescence

Kidney Pathology - Obsolescence	Replagal (n=12)	Placebo (n=9)
Baseline - Mean±SE	0.191 ± 0.071	0.105 ± 0.051
Week 24 - Mean±SE	0.195 ± 0.060	0.130 ± 0.051
Change from Baseline - Week 24 Mean ± SE	0.004 ± 0.050	0.024 ± 0.034
p-value	0.870	

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